SLOVENSKI STANDARD
SIST EN 16206:2012

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

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

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

Aliments pour animaux - Dosage de l'arsenic par spectrométrie d'absorption atomique par génération d'hydrures (SAAGH) après digestion sous pression par micro-ondes (Extraction à l'acide nitrique à 65 % et au peroxyde d'hydrogène à 30 %)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 16206:2012) 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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.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 total arsenic in animal feeding stuffs by hydride generation atomic absorption spectrometry (HGAAS) after microwave pressure digestion. The limit of quantification is 0,5 µg/l of the test solution. Using a test portion of 0,5 g, a volume of the test solution of 25 ml and an aliquot of 5 ml for pre-reduction the limit of quantification is 0,125 mg/kg in the feed material.

NOTE For feed materials containing organic arsenic species from compounds of marine origin (i.e. arsenobetaine and tetramethylarsine oxide) a higher digestion temperature of the microwave system up to 300 °C may be necessary in order to enable the hydridisation of these arsenic compounds and in order to determine all different kinds of arsenic species in the corresponding feeding stuffs. Alternatively, the digestion procedure of Annex C can be used if the microwave system does not reach higher temperatures up to 300 °C to ensure complete mineralization for HGAAS determination.

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

Arsenic is determined in the test solution by hydride generation atomic absorption spectrometry (HGAAS) 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.

Arsenic ions of the test solution are reduced with a potassium iodide/ascorbic acid solution and hydrochloric acid to arsenic (III) and converted to arsenic hydride (AsH_3) by sodium borohydride. Arsenic hydride is transferred by a gas stream into a heated measurement cell and decomposed. The absorption at the arsenic line at 193,7 nm corresponds to the amount of arsenic.

Since arsenic (III) and arsenic (V) show a different sensitivity with the hydride technique, it is necessary to reduce arsenic (V) to arsenic (III) in order to avoid incorrect measurements.

Other digestion procedures with the same digestion efficiency are possible in order to completely mineralize all arsenic species like organic arsenic species from compounds of marine origin for HGAAS determination (see Annex C).

NOTE 1 When using e.g. perchloric acid as alternative digestion procedure to ensure complete mineralisation of all organic and inorganic arsenic species for HGAAS determination you must use NaI/L-ascorbic acid because KI results in precipitation of potassium perchlorate.

NOTE 2 Alternatively, inductively-coupled-plasma mass-spectrometry (ICP-MS) for measuring can be used where an incomplete mineralization is not of importance.

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 arsenic in the reagents and apparatus used.

Use water conforming to grade 2 of EN ISO 3696.

NOTE High purity is essential to avoid potential contamination. Therefore, only use reagents available with high purity or perform a digestion 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 Hydrogen peroxide, not less than 30 % (mass fraction), of approximately ρ (H_2O_2) ≥ 1,1 g/ml.

4.3 Hydrochloric acid, ≥ 30 % (mass fraction), of approximately ρ (HCl) ≥ 1,15 g/ml.

4.4 Diluted hydrochloric acid, e.g. about 3 % (mass fraction), used as carrier solution in the flow injection procedure and for dilution of the arsenic stock solution to the 1 mg/l standard solution and furthermore to the calibration solutions.

EXAMPLE Dilute approximately 90 ml of hydrochloric acid (4.3) to 1 l with water.

4.5 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.

4.6 Potassium iodide/ascorbic acid solution.

Dissolve 2,5 g of potassium iodide and 2,5 g of L-ascorbic acid in water and dilute to 100 ml. Prepare a fresh solution on the day of the analysis.

NOTE The concentrations of the potassium iodide and ascorbic acid may vary slightly with the system and the instructions of the relevant manufacturer shall therefore be observed.

4.7 Arsenic stock solution, c (As) = 1 000 mg/l.

Stock solutions are commercially available. It is advisable to use certified stock solutions. Otherwise dissolve 1,320 g of diarsenic trioxide (As_2O_3) in 25 ml of potassium hydroxide solution (c = 20 g/100 ml), neutralize with 20 % (mass fraction) sulfuric acid with phenolphthalein as indicator and dilute to 1 000 ml with 1 % (mass fraction) sulfuric acid.

4.8 Arsenic standard solution, c (As) = 1 mg/l.

Pipette, for example, 100 µl of the stock solution (4.7) into a 100 ml flask (5.3) and fill up with hydrochloric acid (4.4) to reach a concentration of 1 mg/l.

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

4.9 Arsenic calibration solutions.

For the preparation of five calibration solutions the following procedure is recommended: Dilute 0 ml, 1,25 ml, 2,5 ml, 7,5 ml and 12,5 ml of the arsenic standard solution (4.8) with hydrochloric acid (4.4) into 50 ml flasks (5.3) and mix thoroughly. Then pipette 1 ml of each solution into 25 ml flasks (5.3), add 2,5 ml potassium iodide/ascorbic acid solution (4.6) and 2,5 ml of hydrochloric acid (4.3), mix thoroughly, and let the solutions stand at room temperature for 60 min. Finally make up to the mark with hydrochloric acid (4.4) and wait again 60 min at room temperature before the calibration solutions are measured (see Table 2). The concentrations of the calibration solutions are: 0 µg/l, 1 µg/l, 2 µg/l, 6 µg/l and 10 µg/l (see Table 1).

Table 1 — Calibration solution concentrations (4.9) after pre-reduction

Arsenic (As)	**Concentration of calibration solution (4.9) after pipetting 1 ml from the 50 ml flasks (5.3) into 25 ml flasks (5.3) for pre-reduction** µg/l	**Aliquot of arsenic standard solution (4.8) transferred in 50 ml flasks (5.3)** ml
Calibration standard 1	0	0
Calibration standard 2	1	1,25
Calibration standard 3	2	2,50
Calibration standard 4	6	7,50
Calibration standard 5	10	12,5

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 the electronics should be especially 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, 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 Specific lamp for arsenic.

NOTE An electrode less discharge lamp (EDL) is preferred 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 arsenic and for other minerals and trace elements which correspond to the total contents of these elements. For some specific problems, like incomplete mineralization of organic arsenic in marine compounds, 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 an accuracy of 1 mg for digestion.

Add e.g. 5 ml nitric acid (4.1) and 2,5 ml hydrogen peroxide (4.2) 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 the addition of nitric acid and hydrogen peroxide. Therefore, let the reactions fade off at room temperature, i.e. over night.

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.

WARNING 2 — For samples with unknown composition, firstly carry out a digestion procedure with a low test portion. In particular cases heavy reactions with hydrogen peroxide could appear. In addition, formation of highly explosive compounds is possible when organic matrices are digested. Too high weights could result in uncontrollable reactions.