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BSI Standards Publication

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)

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National foreword

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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)

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 %)

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

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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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.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.

In principle, the pressure digestion is started with low power then continuously increased to the maximum permitted power supply for a distinct time to achieve a temperature of more than 200 °C. The digestion requires about 15 min to 30 min. Afterwards, the system is cooled down.

NOTE 3 With a digestion temperature of 200 °C a sufficient digestion of arsenic (and other elements) is obtained. In general, it applies that the quality of the digestion will become better with increasing digestion temperature. Digestion temperatures up to 300 °C can be necessary in this procedure for a complete mineralization of all organic arsenic species from compounds of marine origin otherwise too low results of total arsenic may occur (see Table A.1 – fish feed).

Remove the reaction vessels from the microwave system in an extractor hood or a fume cupboard and let them release the pressure carefully. Let the vessels stand opened for about 20 min to pass off brown (nitrose) gases. The use of an ultrasonic bath or a water bath with a water temperature of about 80 °C (5.7) is recommended to degas the digestion solution.

When loosing reaction gases within the microwave digestion the whole digestion procedure is to be repeated with a reduced test portion; this is very obvious when the volume of the digestion solution is reduced after the pressure digestion procedure.

Finally, when the digestion solution has achieved room temperature it is quantitatively transferred to a 25 ml flask or 50 ml flask (5.3) and filled up to the mark with water. For graduated reaction vessels, the digestion solution could directly be filled up to the mark with water.

The digestion solution should be clear. When there are suspended particles in the digestion solution, allow them to settle or else filter or centrifuge the solution before transferring it to a vessel of PTFE, PFA or FEP. If the measuring is not done immediately after the digestion, the test solution shall be stored in suitable vessels to prevent a loss or a carryover of arsenic.

6.3 Measurement of the test solution

6.3.1 Pre-reduction of arsenic (V) to arsenic (III).

6.3.2 Pre-reduction of the (pre-diluted) test solution

Transfer 1 ml of the test solution (6.2) to a 25 ml flask (5.3), add 2,5 ml potassium iodide/ascorbic acid solution (4.6) and 2,5 ml hydrochloric acid (4.3) and mix thoroughly. Let the solutions stand at room temperature for 60 min. Finally, make up to the mark with hydrochloric acid (4.4) and leave again for 60 min at room temperature before the test solutions are measured (Table 2).

Table 2 — Recommended procedure for the pre-reduction of test solutions to measure arsenic by flow-injection HGAAS

Flask (volume of test solution after pressure digestion)	**25 ml – 100 ml**
Aliquot of test solution (6.2) [a]	**1 ml to 5 ml (6.2)**
Flask for pre-reduction (final volume)	**25 ml**
Potassium iodide/ascorbic acid solution (4.6)	**2,5 ml**
Hydrochloric acid (4.3)	**2,5 ml**
Incubation time	**60 min**
Temperature	**Room temperature**
Fill up with:	**Diluted hydrochloric acid (4.4)**
Incubation time	**60 min**
Dilution factor for pre-reduction	**1:25 (if aliquot of the test solution = 1 ml)**
[a] The aliquot of the test solution could be reduced or increased, depending on the concentration of arsenic in the test sample.	

6.3.3 Settings of the atomic absorption spectrometer (HGAAS - procedure)

To devise a test schedule, first adjust the apparatus as specified in the operating manual of the manufacturer, then optimise the settings, paying particular attention to gas flow times and the amounts of sodium borohydride introduced. Typical settings are listed in Table 3.

Table 3 — Typical settings of HGAAS for measuring arsenic

Temperature of the cell	**900 °C**
Wave length	**193,7 nm**
Slit width	**0,7 nm**
Signal processing [a]	**Peak height with background correction**
Smoothing	**0,5 s**
Integration time	**15 s**
[a] Nearest to the limit of quantification a signal processing by peak area is recommended.	

6.3.4 HGAAS determination.

The pre-reduced test solutions (6.3.1) and arsenic calibration solutions (4.9) are measured directly with an atomic absorption spectrometer with electrically heated quartz cell coupled to a flow-injection-hydride-system. Use of a 500 µl sample loop is recommended.

The apparatus should be programmed in such a way that first the sample loop is filled with the pre-reduced test or pre-reduced calibration solution. Then the test- or calibration solution is transferred to a mixing unit with diluted hydrochloric acid (4.4) and mixed with sodium borohydride solution (4.5). The resulting gas/liquid mixture is separated by an argon-flowed separator. The argon stream transfers the metal hydrides to the quartz cell for atomisation reaction and measurement of the atomic absorption of arsenic.

Firstly, the arsenic calibration solutions (4.9) are measured, then the pre-reduced test solutions (6.3.1).

Check the linear range of the calibration function. If the concentration of the test solution is outside the linear range, dilute with hydrochloric acid (4.4) and not with pure water. When carrying out prolonged series of measurements, it is advisable to check the zero and the calibration at intervals.

As an analytical control, reference samples having reliable known arsenic contents shall be analysed parallel to all the series of samples analysed, the reference samples being subjected to all the steps in the method starting from digestion. Blank solutions prepared by subjecting them to all steps in the method shall also be determined.

7 Calculation

In general, the calibration curve and the element concentration of the test solution is calculated by the AAS system itself.

The arsenic mass fraction in mg/kg of the weighed test sample (W_s) is calculated according to the following formula:

$$W_s = \frac{(c_t - c_b) \times V_1 \times V_3 \times D}{V_2 \times m \times 1000} \quad (1)$$

where

c_t is the concentration of arsenic in the test solution, µg/l;

c_b is the concentration of arsenic in the blank solution, µg/l;

m is the mass of test portion, g;

V_1 is the volume of test solution after microwave digestion procedure (i.e. 25 or 50), ml;

V_2 is the volume (aliquot) of test solution after microwave digestion procedure (i.e. 1 to 5), ml;

V_3 is the volume of the flask for pre-reduction (i.e. 25), ml;

D is the dilution factor (i.e. 1 when no further dilution is done);

1 000 is the constant factor to calculate from arsenic concentration expressed in µg/ml to µg/l.

EXAMPLE Using m = 0,5 g as test portion, a 25 ml flask (5.3) for microwave digestion (= V_1), an aliquot of 5 ml after digestion (= V_2) and a 25 ml flask (5.3) for the pre-reduction step (= V_3) - the arsenic mass of the sample (= W_s) is calculated as mg/kg in the feed material when no further dilution (D = 1) is done:

$$W_S = (c_t - c_b) \times (25 \times 25 \times 1) / (0{,}5 \times 5 \times 1\,000) = (c_t - c_b) \times 625 / 2\,500 = (c_t - c_b) / 4 \quad mg/kg$$

8 Precision

8.1 Introduction

An inter-laboratory study was organized by Technische Universität München, Research Center for Nutrition and Food Sciences, Bioanalytic Weihenstephan in 2009/2010. The results of the main method protocol using pressure digestion and HGAAS determination are given in Annex A. Results of other digestion procedures (Annex C) and/or other systems like ICP-MS which were alternatively used in this inter-laboratory study were compliant with those of the main protocol, details were given only in the final report [11].

8.2 General

Details of an inter-laboratory test done in 2009/2010 on the precision of the method are summarised in Annex A. The values derived from this inter-laboratory test may not be applicable to concentration ranges and matrices other than those given.

8.3 Repeatability

The absolute difference between two independent single test results, obtained with 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 no more than 5 % of the cases exceed the values of r given in Table 4.

8.4 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, will in no more than 5 % of the cases exceed the values of R given in Table 4.

Table 4 — Precision data

Matrix	Mean mg/kg	r mg/kg	R mg/kg
Turkey feed	0,100	0,027	0,058
Hay	0,220	0,053	0,096
Mineral piglet feed	2,01	0,23	0,64
Grass silage	3,58	0,43	0,81
Bentonite Montmorillonite	6,44	0,78	2,69
Fish feed	3,31	0,65	1,00

9 Test report

The test report shall specify the following information:

a) information necessary for complete identification of the sample;

b) the test method used, with reference to this European Standard;

c) the test results obtained and the units in which they are specified;

d) date of sampling and sampling procedure (if known);

e) date when the analysis was finished;

f) operating details not specified in this European Standard, or regarded as optional, together with details of any incidents that occurred when performing the method that may have influenced the test result(s).

Annex A
(informative)

Results of the inter-laboratory test

Table A.1 — Precision data

NOTE Fish feed contains 3 mg/kg organic arsenic species which was not detected by 11 labs because they used a microwave system with a digestion temperature of 200 °C - instead of 300 °C described in the protocol - and getting poor results for total arsenic of only 0,3 mg/kg. Therefore, the results of these non compliant labs are not accepted.

Matrix tested	**Turkey feed**	**Hay**	**Mineral piglet feed**	**Grass silage**	**Bentonite Mont-morillonite**	**Fish feed**	**Turkey feed**	**Mineral piglet feed**
Subsamples fully prepared for direct weighing	yes	yes	yes	yes	yes	yes	no	no
No. of labs	18	18	20	20	20	18	18	20
No. of outlier labs	2	3	5	4	2	0	3	2
No. of non compliant labs	0	0	0	0	0	11	0	0
No. of valid labs	16	15	15	16	18	7	15	18
Mean value, mg/kg	0,100	0,220	2,01	3,58	6,44	3,31	0,101	1,94
s_r, mg/kg	0,010	0,019	0,08	0,15	0,28	0,23	0,009	0,07
r, mg/kg	0,027	0,053	0,23	0,43	0,78	0,65	0,025	0,21
RSD(r), %	9,6	8,6	4,1	4,3	4,3	7,0	8,9	3,8
S_R, mg/kg	0,021	0,034	0,23	0,29	0,96	0,36	0,017	0,26
R, mg/kg	0,058	0,096	0,64	0,81	2,69	1,00	0,047	0,73
RSD(R), %	20,6	15,7	11,3	8,1	14,9	10,7	16,5	13,4
HORRAT(R)	0,9	0,8	0,8	0,6	1,2	0,8	0,7	0,9

Annex B
(informative)

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

Weigh test portions of 0,2 g to 0,5 g in reaction vessels of the microwave pressure digestion system, considering the part of organic matter of the sample and the size of the reaction vessel.

↓

Add 5 ml nitric acid (65 %) and 2,5 ml hydrogen peroxide (30 %) using reaction vessels of 100 ml size.

↓

Digest using an adequate temperature program considering the manufacturers manuals.

NOTE Digestion temperature should achieve 200°C - for organic arsenic species of compounds from marine origin up to 300°C.

↓

Cool down the reaction vessels, release pressure carefully, then open and let pass off brown (nitrose) gases by using an ultrasonic or a water bath.

↓

Transfer quantitatively the digestion solution to a graduated flask (not necessary when reaction vessels are graduated) and fill up with water. Filter or centrifuge the test solution or allow particles to settle, if necessary.

↓

Transfer the clear test solution to vials of PTFE, PFA or FEP for measuring arsenic by HGAAS (or ICP-MS).

↓

Dilute the arsenic stock solution to a standard solution and prepare at the day of analysis 5 pre-reduced calibration solutions with potassium iodide/ascorbic acid solution and hydrochloric acid (37 %) and wait for 60 min. Dilute with hydrochloric acid (3 %), wait again for 60 min before measuring.

↓

Take an aliquot of the test solution for pre-reduction with potassium iodide/ascorbic acid solution and hydrochloric acid (37 %) and wait for 60 min. Dilute with hydrochloric acid (3 %), wait again for 60 min before measuring.

↓

Prepare the reduction and the carrier solution and adjust the AAS system as specified in the operating manual of the manufacturer and optimise the typical settings.

↓

Measure the calibration, test, blank and reference solutions at 193,7 nm by flow-injection HGAAS. Calculate the sample results considering the reference and blank samples for control.

Annex C
(informative)

Alternative digestion procedure with the same digestion efficiency to ensure complete mineralization of all organic and inorganic arsenic species for HGAAS measurement: dry ashing with magnesium oxide and magnesium nitrate as ashing reagents

C.1 Arsenic calibration solutions

For the preparation of calibration solutions the following procedure is recommended: Add 0 ml, 1 ml, 2 ml, 5 ml and 10 ml of a 100 µg/l arsenic standard solution into 100 ml flasks. Add 50 ml 3 mol/l hydrochloric acid and 5 ml 20 % potassium iodide/20 % ascorbic acid solution and mix thoroughly. Fill the flask to 100 ml with 3 mol/l hydrochloric acid Let the calibration solutions stand at room temperature for 30 min.

The concentrations of the calibration solutions are: 0 µg/l, 1 µg/l, 2 µg/l, 5 µg/l and 10 µg/l.

C.2 Preparation of the test solution

Weigh 2 g test portion in a quartz dish and add 3 g of magnesium oxide and 20 ml of 10 % magnesium nitrate solution and mix. The mixture is dried at 100 °C and ashed at 575 °C overnight. Dissolve the ash with 50 ml 6 mol/l hydrochloric acid, heat and filter the solution into a 100 ml flask. Rinse the crucible, funnel and filter paper thoroughly with hot water until 90 ml filtrate is collected. Cool the solution and fill the flask to 100 ml. The solution is now ready for the pre-reduction procedure. The acid concentration of the test solution is 3 mol/l hydrochloric acid.

C.3 Measurement of the test solution

C.3.1 Pre-reduction of arsenic (V) to arsenic (III)

Add 1 ml - 25 ml test solution to a 50 ml flask depending on the needed dilution. Add 2,5 ml 20 % potassium iodide/20 % ascorbic acid solution and fill the flask with 3 mol/l hydrochloric acid. Let the solution stand for 30 min.

C.3.2 Atomic absorption spectrometer (HGAAS-procedure)

The test solution is measured with HGAAS as described in 6.3.3.

NOTE The procedure in Annex C ensures complete mineralization of all organic and inorganic arsenic species for the determination of total arsenic contents in animal feeding stuffs by HGAAS [9].

Bibliography

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[4] EN 14627, *Foodstuffs — Determination of trace elements — Determination of total arsenic and selenium by hydride generation atomic absorption spectrometry (HGAAS) after pressure digestion*

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