SLOVENSKI STANDARD
SIST EN 16278:2012

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Živalska krma - Določevanje anorganskega arzena s hidridno atomsko absorpcijsko spektrometrijo (HG-AAS) po mikrovalovni ekstrakciji in ločevanju z ekstrakcijo na trdni fazi (SPE)

Animal feeding stuffs - Determination of inorganic arsenic by hydride generation atomic absorption spectrometry (HG-AAS) after microwave extraction and separation by solid phase extraction (SPE)

Futtermittel - Bestimmung von anorganischem Arsen mit Atomabsorptionsspektrometrie-Hydridtechnik (HD-AAS) nach Mikrowellen-Extraktion und Trennung durch Festphasenextraktion (SPE)

Aliments des animaux - Dosage de l'arsenic inorganique par spectrométrie d'absorption atomique par génération d'hydrures après extraction par micro-ondes

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COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 16278:2012) 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of an identical text or by endorsement, at the latest by January 2013, and conflicting national standards shall be withdrawn at the latest by January 2013.

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1 Scope

This European Standard describes a procedure for the determination of inorganic arsenic in animal feeding stuffs of marine origin by Solid Phase Extraction (SPE) and Hydride Generation Atomic Absorption Spectrometry (HG-AAS). The method has been successfully tested in a collaborative trial with a working range from 0,19 mg/kg to 2,7 mg/kg (HORRATvalues < 2; HORRAT value is Horwitz –Ratio value). The LOQ of the method is usually approximately 0,1 mg/kg or lower.

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)*

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

3 Principle

Inorganic arsenic consists of arsenite, As(III) and arsenate, As(V). This standard describes a method for the determination of inorganic arsenic (i.e. the sum of As(III) and As(V)). A representative test portion of the sample is treated with a diluted hydrochloric acid and hydrogen peroxide solution using microwave assisted heating. Hereby inorganic arsenic species are extracted and As(III) is oxidised to As(V). The inorganic arsenic is selectively separated from other arsenic compounds using solid phase extraction (SPE) and the concentration of inorganic arsenic is determined by HG-AAS in the SPE eluate. The gaseous hydride is transferred into a heated measuring cell (cuvette) by means of a carrier gas stream and decomposed.
Since arsenic (III) and arsenic (V) show a different sensitivity to the hydride technique, it is necessary to reduce arsenic (V) to arsenic (III) in order to avoid incorrect measurements.

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 its use.

4 Reagents

The concentration of arsenic in the reagents and water used shall be low enough not to affect the results of the determination. All reagents shall be of analytical grade or similar unless otherwise specified.

Use water conforming to grade 2 of EN ISO 3696.

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

NOTE Only use hydrochloric acid available with high purity or which was cleaned by a sub-boiling distillation.

4.2 Diluted hydrochloric acid.

4.2.1 0,4 mol/l as eluent for the SPE.

Dilute e.g. 20 ml of hydrochloric acid (4.1) to 0,5 l with water.

4.2.2 0,055 mol/l for the extractant solution.

Dilute e.g. 5,8 ml of hydrochloric acid (4.1) to 1 l with water.

4.2.3 2,6 mol/l for sample pre-reduction.

Dilute e.g. 270 ml of hydrochloric acid (4.1) to 1 l with water.

4.2.4 4,7 mol/l for HG-AAS measurements.

Dilute e.g. 500 ml of hydrochloric acid (4.1) to 1 l with water.

4.3 Hydrogen peroxide (H_2O_2), mass fraction not less than 30 %.

4.4 Extractant solution, volume fraction of 3 % H_2O_2 in 0,055 mol/l HCl.

Dilute e.g. 20 ml hydrogen peroxide (4.3) to 200 ml with 0,055 mol/l hydrochloric acid (4.2.2).

4.5 Acetic acid, ρ (CH_3COOH) = 1,05 g/ml.

4.6 Diluted acetic acid, 0,5 mol/l for washing of SPE columns.

Dilute e.g. 15 ml of acetic acid (4.5) to 0,5 l with water.

4.7 Sodium hydroxide pellets, mass fraction of ≥ 98 %.

4.8 Sodium hydroxide, 0,1 mol/l for adjustment of pH.

Dilute 0.4 gram sodium hydroxide pellets (4.7) to 100 ml with water.

4.9 Ammonium carbonate, mass fraction of ≥ 99,999 %.

4.10 Ammonium carbonate buffer solution, 40 mmol/l for sample buffering.

Dissolve e.g. 0,384 g ammonium carbonate (4.9) in water and dilute to 100 ml with water.

4.11 Methanol (CH_3OH), HPLC grade, for conditioning of SPE sorbent.

4.12 Sodium borohydride, mass fraction of ≥ 96 %.

4.13 Sodium borohydride solution for hydride generation.

Dissolve e.g. 2,5 g of sodium hydroxide pellets (4.7) in a small amount of water; add 2,5 g of sodium borohydride (4.12) and dilute to 500 ml with water. Prepare a fresh solution daily prior to the hydride generation step and, when necessary, filter before use.

Commercially available sodium borohydride solutions may also be used.

NOTE The required concentrations may vary slightly with the system and the instructions of the relevant manufacturer shall therefore be observed.

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 the measurements are carried out.

4.14 L-Ascorbic acid, mass fraction of ≥ 99,7 %.

4.15 Potassium iodide, mass fraction of ≥ 99,5 %.

4.16 Pre-reduction solution for sample extract.

Dissolve 1,25 g of potassium iodide (4.15) and 1,25 g of L-ascorbic acid (4.14) up to 250 ml with 2,6 mol/l hydrochloric acid (4.2.3). Prepare a fresh solution on the day of analysis.

The required concentrations may vary slightly with the system and the instructions of the relevant manufacturer shall therefore be observed.

NOTE Addition of a volume fraction of 0,1 % silicone (4.21) to the solution can reduce the formation of foam in the gas-liquid separation system.

4.17 SPE equilibration solution.

Mix equal volumes (1+1) of 40 mmol/l ammonium carbonate solution (4.10) with extractant solution (4.4). 2 ml per SPE cartridge is needed.

4.18 Arsenic stock solution.

Commercially available standards with a mass concentration of 1 000 mg/l are recommended. Stock solutions in diluted nitric acid are preferred.

4.19 Diluted inorganic arsenic solutions.

Dilute the arsenic stock solution (4.18) by water in several steps, such as in the following example:

$$1\ 000\ \text{mg/l} \xrightarrow[1/100]{} 10\ \text{mg/l} \xrightarrow[1/10]{} 1\ \text{mg/l} \xrightarrow[1/10]{} 0{,}1\ \text{mg/l}$$

4.20 Calibration solutions.

Prepare six external standards including a blank calibration solution that covers the linear range of the element to be determined by diluting the diluted inorganic arsenic solutions (4.19). The linear range is instrument dependent. Appropriate matching of the calibration solutions shall be performed (prereduction according to Table 2). It is important to adjust the acid concentration of the standards to the acid concentration of the samples.

The following calibration range is recommended: 0,25 µg/l to 8 µg/l. These calibration solutions should be prepared freshly before use.

NOTE An example on the production of calibration standard is given in Annex B, Table B.2.

4.21 Silicone antifoam agent ≥ 30 %.

5 Apparatus and equipment

To minimise contamination, all apparatus and equipment that come into direct contact with the sample and the solutions shall be carefully pre-treated. It is recommended to avoid the use of glassware, since this may cause contamination with arsenate [1].

5.1 Laboratory grinder, capable of grinding to a particle size of less than or equal to 0,5 mm.

5.2 Analytical balance, capable of weighing to an accuracy of 1 mg.

5.3 Microwave oven with closed vessels, capable of programming temperature.

5.4 pH indicator paper/sticks, for pH range 4 to 7.

5.5 Centrifuge with containers, capable of a minimum centrifuge speed of 4 000 rpm (2 100 *g*).

5.6 Solid phase extraction cartridges, with strong anion exchange stationary phase.

Strata SAX (Phenomenex), 500 mg / 6 ml have proven suitable for the present procedure. (Equivalent SPE columns with similar performance from other producers may also be used.)

NOTE Check the capacity of the SPE cartridges for retainment of inorganic arsenic.

5.7 Vacuum chamber or similar, for the elution process of the SPE.

5.8 One-mark volumetric flasks, of the following sizes: 100 ml, 150 ml, 250 ml, 500 ml, 1 000 ml.

5.9 Atomic absorption spectrometer, with measurement recording system, heated quartz cell and accessories for the hydride generation method.

5.10 Element-specific lamp, (electrode less discharge lamp (preferred) or hollow-cathode) for arsenic.

5.11 Automated flow system for hydride generation.

6 Procedure

6.1 General

IMPORTANT — The procedure is described in a flowchart (Annex B). Furthermore, Table B.1 provides information on which solutions and equipment are used for the different sections of the method: µ - wave, SPE and HG-AAS, respectively.

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

6.2 Microwave assisted extraction

A test portion of approximately 0,2 g of the homogenised and ground (to a particle size of < 0,5 mm) test sample is weighed to an accuracy of 1 mg into a microwave oven vessel. 10,0 ml of the extractant solution (4.4) is added. The solution is then subjected to microwave-assisted heating using the temperature program outlined in Table 1.

Table 1 — Microwave oven programme

Step	Temperature	Time
1	90 °C	25 min
2	Cooling	7 min (time may vary; follow manufacturer's instructions)

Following microwave treatment, the samples are allowed to cool to room temperature. The supernatant is then transferred to centrifuge containers and centrifuged for 10 min at ≥ 4 000 rpm (2 100 *g*).

NOTE The supernatant is used for subsequent SPE clean up and can be stored in clean containers at 4 °C for a maximum of three weeks until usage.

6.3 Solid phase extraction of inorganic arsenic

IMPORTANT — For adding liquids to the SPE cartridges, a low dropping speed is important. For adding a volume of 2 ml liquid, approximately 5 min elution time is recommended.

6.3.1 Sorbent conditioning of SPE cartridges

The SPE cartridges are conditioned with 2 ml methanol (4.11).

IMPORTANT — Let the SPE cartridges run dry.

6.3.2 Sorbent equilibration of SPE cartridges

The SPE cartridges are conditioned with 2 ml SPE sorbent equilibration solution (4.17).

IMPORTANT — Let the SPE cartridges run dry.

6.3.3 Buffering of sample solution

3 ml of the sample supernatant (6.2) is mixed with 3 ml 40 mmol/l ammonium carbonate buffer solution (4.10). Ensure the pH of the solution is 6,5 ± 1 with pH indicator paper/sticks (5.4). Adjust if necessary with diluted acetic acid (4.6) or diluted sodium hydroxide (4.8).

Centrifuge the buffered sample solution at > 4 000 rpm (2 100 *g*) for at least 10 min to remove particles.

6.3.4 Loading of sample solution

4 ml of the centrifuged buffered sample solution (6.3.3) is loaded to the SPE cartridges.

IMPORTANT — Let the SPE cartridges run dry.

6.3.5 Washing of SPE cartridges

Wash the SPE cartridges with 3 ml 0,5 mol/l acetic acid (4.6).

IMPORTANT — Let the SPE cartridges run dry, using vacuum (50 kPa to 70 kPa) for at least 5 min before the final elution step (6.3.6).

6.3.6 Elution of SPE cartridges

The retained As(V) on the SPE cartridge is eluted with 1,25 ml of 0,4 mol/l hydrochloric acid (4.2.1).

IMPORTANT — Collect all sample solvent in suitable plastic containers by applying suitable vacuum (usually in the range of 50 kPa to 70 kPa) for at least 5 min.

NOTE The eluate (6.3.6) can usually be stored at maximum 4 °C for a maximum of three days until analysis.

6.4 Determination of inorganic arsenic

6.4.1 General

The inorganic arsenic is determined as the total amount of arsenic in the SPE eluate. Carry out pre-reductions of test solutions (6.3.6) and calibration solutions (4.20) prior to the determination.

6.4.2 Pre-reduction

This step is dependent on the hydride system used. Therefore, please follow the optimised hydride generation procedure of the equipment used and note that it may be necessary to use larger or smaller volumes than described below (Table 2).

Table 2 — Example of a procedure for the pre-reduction of test solutions prior to HG-AAS measurement

Aliquot of test solution (6.3.6)	1 ml
Potassium iodide/ascorbic acid solution with hydrochloric acid (4.16)	Add 7 ml and mix thoroughly
Incubation	60 min at room temperature
2,6 mol/l hydrochloric acid (4.2.3)	Add 6 ml and mix thoroughly
Incubation	60 min at room temperature

All samples including the calibration solutions should be treated in the same way. The hydrochloric acid (4.2.3) and reducing-agent (4.16) concentrations shall be the same in all the test solutions (see Annex B, Table B.2 for an example).

6.4.3 Settings of the Atomic absorption spectrometer (HG-AAS 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 amount of sodium borohydride introduced. Typical settings are listed in Table 3.

Table 3 — Typical settings of HG-AAS for arsenic measurement

Temperature of the cell	900 °C
Wave length	193,7 nm
Slit width	0,5 nm
Signal processing	Peak height with background correction
Measurement time	4,0 s

6.4.4 HG-AAS determination

The pre-reduced test solutions and pre-reduced calibration solutions are measured directly with an atomic absorption spectrometer with electrically heated quartz cell coupled to an automated flow system (5.11). The apparatus should be programmed to mix appropriate amounts of test- or calibration solution (6.4.2), diluted hydrochloric acid (4.2.4) and borohydride solution (4.13). The resulting gas/liquid mixture is separated by an argon-flow separator. The argon stream separates and transports the arsenic hydrides to the quartz cell for atomisation reaction and measuring the atomic absorption of arsenic.

The pre-reduced calibration solutions are measured first; then the test solutions are measured. Reanalyse the calibration solutions at the end of each analytical series.

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