SLOVENSKI STANDARD
oSIST prEN 17374:2019

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Krma: metode vzorčenja in analize - Določevanje anorganskega arzena v krmi z anionsko izmenjavo HPLC-ICPMS

Animal feeding stuffs: Methods of sampling and analysis - Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICPMS

Futtermittel - Probenahme- und Untersuchungsverfahren - Bestimmung von anorganischem Arsen in Futtermittel mittels Anionenaustausch HPLC-ICPMS

Aliments des animaux - Méthodes d'échantillonnage et d'analyse - Détermination de la teneur en arsenic inorganique dans les aliments pour animaux, par CLHP avec échange d'anions et spectrométrie de masse à plasma induit par haute fréquence (ICP-SM)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (prEN 17374:2019) 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 document is currently submitted to the CEN Enquiry.

This document has been prepared under a standardization request given to CEN by the European Commission and the European Free Trade Association.

WARNING — The method described in this standard implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this standard to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

1 Scope

This method procedure describes a procedure for the determination of inorganic arsenic in animal feeding stuffs by anion-exchange HPLC-ICP-MS following water bath extraction.

This method was successfully tested in the range of 0,149 mg/kg to 9,69 mg/kg in the following animal feed matrices: rice meal, seaweed meal, fish meal, grass meal, complete feed (marine-based), complete feed (cereal based) and a synthetic solution.

NOTE Mineral feed matrices are not included in the scope of this method as the determination of the total arsenic content is more suitable in such matrices.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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)*

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

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

3 Principle

This standard describes a method for the determination of inorganic arsenic in animal feeding stuffs. Inorganic arsenic consists of arsenite, As(III) and arsenate, As(V). A representative test portion of the sample is treated with a diluted nitric acid and hydrogen peroxide solution in a heated waterbath. Hereby the arsenic species are extracted into solution and As(III) is oxidized to As(V). The inorganic arsenic is selectively separated from other arsenic compounds using anion exchange HPLC (High Performance Liquid Chromatography) coupled online to the element-specific detector ICP-MS (Inductively Coupled Plasma Mass Spectrometry) for the determination of the mass fraction of inorganic arsenic. External calibration with solvent standards is used for quantification of the amount of inorganic arsenic.

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4 Reagents

Use only reagents of recognized analytical grade and water conforming to grade 1 of ISO 3696.

4.1 General

The concentration of arsenic species in the reagents and water used shall be low enough to not affect the results of the determination. Reagents should be of minimum p.a. quality where possible. Special facilities should be used in order to avoid contamination during the steps of preparation and measurement (e.g. uses of laminar flow benches or comparable clean facilities).

NOTE When using a method of high sensitivity like ICP-MS, the control of the blank levels of water, acid and other reagents is very important. Generally ultra-pure water complying with ISO 3696 grade 1 (i.e. electrical conductivity below 0,1 µS/cm at 25 °C) and acid of high purity, e.g. cleaned by sub-boiling distillation is suitable.

4.2 Nitric acid (HNO_3), concentrated, ≥ 65 % (mass fraction), mass concentration of approximately ρ (HNO_3) 1,4 g/ml.

Use only nitric acid available with high purity or perform a clean-up by a sub-boiling distillation in order to avoid potential contamination.

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

High purity is essential to avoid potential contamination. Commercially available hydrogen peroxide for analysis should be tested for contamination of arsenic prior to use.

4.4 Extraction solution 1, 0,1 mol/l HNO_3 in 3 %(V/V) H_2O_2

Pour 800 ml of H_2O and then 6,5 ml of HNO_3 (4.2) and thereafter 100 ml of H_2O_2 (4.3) into a 1 000 ml volumetric flask. Fill it up to the mark with H_2O. This solution should be prepared on the same day of use.

Estimate the total volume needed for the analysis and produce only this amount.

4.5 Extraction solution 2, 0,2 mol/l HNO_3 in 6 % H_2O_2

Pour 70 ml of H_2O, 1,3 ml of HNO_3 (4.2) and 20 ml of H_2O_2 (4.3) into a 100 ml volumetric flask. Fill it up to the mark at 100 ml with H_2O. This solution should be prepared on the same day of use.

Estimate the total volume needed for the analysis and produce only this amount.

4.6 Ammonium carbonate, $(NH_4)_2CO_3$, mass fraction w ≥ 99,999 %, for production of mobile phase solution.

4.7 Aqueous ammonia, (NH_3(aq).) *w* ≥ 25 %, for adjustment of pH in the mobile phase.

4.8 Methanol, (CH_3OH), HPLC grade, for production of mobile phase.

4.9 Mobile phase, e.g. 50 mmol/l ammonium carbonate in 3 % methanol at pH 10,3

Dissolve e.g. 4,80 g of ammonium carbonate (4.6) in approximately 800 ml of water. Adjust the pH to 10,3 with aqueous ammonia (4.7) and add 30 ml of methanol (4.8) and then fill up to 1 000 ml with water. Filter the mobile phase solution through a 0,45 µm filter prior to use (4.4).

The optimal concentration of ammonium carbonate in the mobile phase depends on the analytical column used (e.g. brand, particle size and dimensions). The appropriate concentration of ammonia carbonate is in the discretion of the analyst and should fulfil the criteria for sufficient resolution of the arsenate peak as stated in 5.10.

Methanol is added to the mobile phase in order to enhance the signal intensity for arsenic (carbon enhancement effect [1]). The concentration of methanol for maximum signals depends on the instrument used and should be identified by the analyst.

4.10 Diarsenic trioxide, *w*(As_2O_3) ≥ 99,5 %, optional.

4.11 Potassium hydroxide solution, *ρ*(KOH) = 20 g/100 ml, optional.

4.12 Sulfuric acid solutions, *w*(H_2SO_4) = 20 % and w(H_2SO_4) = 1 %, optional.

4.13 Phenolphthalein, optional.

4.14 Standard solutions, with an arsenic mass concentration of 1 000 mg/l.

The use of commercial standards of arsenic, arsenic III and/or V, with a mass concentration of 1 000 mg/l is recommended.

Otherwise proceed as follows: Dissolve e.g. 1,320 g of diarsenic trioxide (4.9) in 25 ml of potassium hydroxide solution (4.10), neutralize with 20 % sulfuric acid solution (4.11) with phenolphthalein (4.12) as indicator and dilute to 1 000 ml in a volumetric flask with 1 % sulfuric acid solution (4.11).

4.15 Calibration solutions

Prepare a range of standards including a blank calibration solution that covers the linear range of the analyte to be determined by diluting the analyte stock solution (4.14) with extraction solution (4.4). Preparation of the calibration solutions shall be performed by using the extraction solution (4.4) for the final dilution step, which will prevent reduction of arsenate to arsenite. Transfer an aliquot of the calibration solutions to HPLC vials prior to analysis.

The quantitative oxidation of arsenite to arsenate in the standard solutions should be verified (e.g. visual inspection of chromatogram by looking for an additional peak or a reduced intensity of the arsenate peak).

NOTE By preparing the standard in the extraction solution 1 (4.4) all arsenite will be completely oxidized to arsenate.

4.16 Solution for checking chromatographic separation, containing the organic arsenic compounds (e.g. 10 µg/l) monomethylarsenous acid (MA), dimethylarsinic acid (DMA) and arsenobetaine (AB), as well as arsenate (e.g. 10 µg/l) and chloride (e.g. 100 mg/l).

5 Apparatus and equipment

5.1 General

To minimize the 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 [2].

WARNING — Some auto sampler systems use syringes made of glass. In this case, you can only check for contamination and minimize it.

5.2 Laboratory grinder, capable of grinding to a particle size of less than 0,3 mm.

5.3 Analytical balance, accuracy of 1 mg.

5.4 Filtering device, for filtration of mobile phase, pore size 0,45 µm.

5.5 Water bath, capable of programming of the temperature at 90 °C.

5.6 Centrifuge, for minimum 4 000 min^{-1} (2 010 g).

5.7 Single use syringe filters (0,45 µm) or HPLC vials with filters, compatible with acidic solutions for filtering of test solutions prior to analysis.

5.8 Plastic volumetric flasks, for preparation of mobile phase and calibration solutions.

NOTE If calibration standards are prepared by weighing, plastic ware without marks are suitable.

5.9 High Pressure Liquid Chromatography (HPLC).

5.10 Strong anion exchange column (SAX), suitable for selective separation of arsenate from other arsenic compounds present in the sample extracts.

Usually, the minimum acceptable retention time for the analyte is twice the retention time corresponding to the void volume of the column. Furthermore, the nearest peak in the chromatogram should be separated from the analyte peak by at least one full peak width at 10 % of the analyte peak height. It is recommended to verify sufficient separation of the analyte peak using a solution of organic arsenic compounds (e.g. monomethylarsenous acid (MA), dimethylarsinic acid (DMA) and arsenobetaine (AB)) and arsenate. Make sure that the HPLC run is long enough for chloride (m/z 35) and for any arsenic compounds with longer retention times than arsenate, to elute from the column prior to injection of the next sample. It should furthermore be ensured that the arsenate and chloride peaks do not co-elute in order to avoid interference from the polyatomic ion $^{40}Ar^{35}Cl^{+}$ in the mass spectrometer.

Use a guard column to prolong the life-time of the analytical column.

5.11 Inductively coupled plasma mass spectrometer (ICP-MS).

5.12 Argon gas, purity ≥ 99,99 %.

6 Sampling

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.

7 Procedure

7.1 Sample preparation

Homogenize the sample using suitable equipment and avoiding excessive heating.

7.2 Water bath extraction

Weigh a test portion of approximately 0,2 g to 0,5 g sample to the nearest milligram, corresponding to dry weight into a tube and fill up to 10,00 ml with extraction solution 1 (4.4). Include also a reagent blank sample. The tubes shall be securely closed with a tight lid. Shake the tubes thoroughly in order to ensure that the sample is wetted sufficiently in the extraction solution 1 (4.4) prior to placing it in the water bath in order to ensure a satisfactory extraction of the analyte. Some finely powdered samples may need extended wetting time (e.g. overnight) prior to the water bath treatment.

If a fresh sample is extracted, the water content has to be taken into account. The sample weight should correspond to 0,2 g to 0,5 g dry matter. The concentration of extraction solution should be adjusted accordingly, keeping the nitric acid concentration at the same level. Proceed e.g. as follows: weigh in the test sample and add water up to 5 ml and mix thoroughly then add double concentrated extraction solution 2 (4.5) to 10 ml and mix.

The solutions are then placed in a heated water bath at 90 °C ± 2 °C and extracted for 60 min ± 5 min.

Following the water bath extraction step, let samples be cooled to room temperature and subsequently centrifuge the tubes (10 min, 4 000 min-1 (2 010 *g*)). The supernatant transferred to clean containers can usually be stored in a refrigerator (at approximately 4 °C) for a maximum of one week until analysis. Prior to analysis, all sample extracts should be filtered (5.7) and transferred to HPLC vials.

7.3 Determination of inorganic arsenic by HPLC-ICP-MS

7.3.1 General

This procedure requires an adequate amount of experience in operating an optimizing the apparatus.

7.3.2 Preparation of apparatus

The HPLC and ICP-MS operating conditions shall be based on the general information provided by the manufacturer of the instruments taking into account the operating conditions of the analytical column.

An arsenic solution, e.g. 10 µg/l arsenate in 3 % methanol, may be used to optimize the test system according to the manufacturer's instructions. Arsenic is mono-isotopic and can be evaluated at a mass/charge ratio (m/z) of 75.

It is advisable to allow the HPLC system (incl. the analytical column) to equilibrate and ensure stable conditions by turning on the HPLC flow in advance prior to start of the analysis. Repeated injections of a sample extract may be necessary until stable chromatography is achieved and the analytical sequence can be started. Depending on the matrix and column condition, retention time shift can occur and should be taken into account.

7.3.3 Calibration

Inject an appropriate volume of the arsenic calibration solutions (4.14) into an anion exchange HPLC-ICP-MS system and determine the peak area of each of the calibration points to construct a calibration curve.

7.3.4 Determination of samples and blank solution

Inject an appropriate volume of the reagent blank solution and the sample test solutions (5.2) into the anion exchange HPLC-ICP-MS and determine the peak areas under appropriate HPLC-ICP-MS settings, e.g. such as listed in Table 1. Test solutions, which give a response outside the linear calibration range, should be diluted appropriately with extraction solution 1 (4.4) to give a response within the linear calibration range. If a significant blank value occurs, identify the source of this blank. The source should be eliminated and the analysis repeated. If the blank is constant and not avoidable, it should be subtracted, see 7.3.

7.3.5 HPLC sequence

Take measures to control the stability of the instrument sensitivity during the analytical run. Control the instrument sensitivity by e.g. analysing a calibration standard solution throughout the sequence (for example, after each five to ten samples) and, if necessary, use the results for re-calibration of the system. Another possibility is to introduce an internal standard (e.g. germanium) post-column (by e.g. a T-split) and use the signal for correction of instrument drift (if any) during the analytical run.

7.3.6 Typical HPLC-ICP-MS settings

Table 1 — Example of typical settings of HPLC-ICP-MS instrumentation

ICPMS settings -	
ICP-MS	Agilent 7500CE
RF power (W)	1 500
Carrier gas flow (l min^{-1})	1,2
Plasma gas flow (l min^{-1})	15
Auxiliary gas flow (l min^{-1})	1,0