
Živalska krma - Določevanje živega srebra z atomsko absorpcijsko spektrometrijo hladnih par (CVAAS) po mikrovalovnem razklopu pod tlakom (ekstrakcija s 65 % dušikovo kislino in 30 % vodikovim peroksidom)

Animal feeding stuffs - Determination of mercury by cold-vapour atomic absorption spectrometry (CVAAS) after microwave pressure digestion (extraction with 65 % nitric acid and 30 % hydrogen peroxide)

Futtermittel - Bestimmung von Quecksilber mit Kaltdampf-Atomabsorptionsspektrometrie (KD-AAS) nach Mikrowellen-Druckaufschluss (Extraktion mit 65 % Salpetersäure und 30 % Wasserstoffperoxid)

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Aliments des animaux - Dosage du mercure par spectrométrie d'absorption atomique à vapeur froide (SAVVF) après digestion sous pression par micro-ondes (extraction avec de l'acide nitrique à 65 % et du peroxyde d'hydrogène à 30 %)

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**Animal feeding stuffs - Determination of mercury by cold-vapour
atomic absorption spectrometry (CVAAS) after microwave
pressure digestion (extraction with 65 % nitric acid and 30 %
hydrogen peroxide)**

Aliments des animaux - Dosage du mercure par
spectrométrie d'absorption atomique à vapeur froide
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(extraction avec de l'acide nitrique à 65 % et du peroxyde
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Atomabsorptionsspektrometrie (KD-AAS) nach
Mikrowellen-Druckaufschluss (Extraktion mit 65 %
Salpetersäure und 30 % Wasserstoffperoxid)

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Foreword

This document (EN 16277:2012) 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 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of an identical text or by endorsement, at the latest by January 2013, and conflicting national standards shall be withdrawn at the latest by January 2013.

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EN 16277:2012 (E)

1 Scope

This European Standard specifies a method for the determination of mercury in animal feeding stuffs by Cold-Vapour Atomic Absorption Spectrometry (CVAAS) after microwave pressure digestion. The limit of quantification in the test solution should be 0,25 µg/l or lower. Using a test portion of 0,5 g and a volume of the test solution of 25 ml a limit of quantification of 0,0125 mg/kg or lower should be obtained.

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/FDIS 6498)*

3 Principle

Mercury is determined in the test solution by cold-vapour atomic absorption spectrometry (CVAAS) after microwave pressure digestion.

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

The test solution is transferred to the reaction vessel of the mercury analysis unit. The mercury is reduced with sodium borohydride or tin(II) chloride to elemental volatile mercury and flushed into the cell of the AAS instrument using a carrier gas stream. As an option with an additional amalgamation step, sensitivity could be increased and matrix effects could be decreased. The absorption at 253,7 nm (mercury line) is used as a measure of the mercury concentration in the cell.

Other digestion procedures with the same extraction efficiency (see Annex D and Annex E) or other measurement systems like FI-CVAAS (flow injection cold-vapour atomic absorption spectroscopy) or CV-ICP-AES (cold-vapour inductively coupled plasma atomic emission spectroscopy) are possible.

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 analysis to control contamination and carry over with mercury in the reagents and apparatus used.

Use water conforming to grade 2 of EN ISO 3696.

4.1 Nitric acid (HNO₃), not less than 65 % (mass fraction), of approximately $\rho(\text{HNO}_3) = 1,4$ g/ml.

NOTE Use nitric acid available with high purity or perform a sub-boiling distillation.

4.2 Hydrogen peroxide (H₂O₂), not less than 30 % (mass fraction).

4.3 Hydrochloric acid (HCl), not less than or equal to 30 % (mass fraction), of approximately $\rho(\text{HCl}) \geq 1,15$ g/ml.

4.4 Diluted hydrochloric acid, e.g. about 3 % (mass fraction), as carrier solution for the use in the flow-injection-system and for dilution of the mercury stock solution to the 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 Reducing agents.

Tin(II) chloride or sodium borohydride may be used as the reducing agent, but it is not advisable to use the two reagents alternately. The concentration by mass of the reducing agent solutions may be varied to suit the system and the relevant information provided by the manufacturer of the apparatus shall be observed.

4.5.1 Tin(II) chloride solution, $c(\text{SnCl}_2 \cdot 2\text{H}_2\text{O}) = 100$ g/l.

Dissolve 50 g of tin(II) chloride in approximately 100 ml of hydrochloric acid (4.3) in a 500 ml volumetric flask (5.2) and dilute to the mark with water. Prepare a fresh solution daily.

4.5.2 Sodium borohydride solution, e.g. $c(\text{NaBH}_4) = 2$ g/l.

Dissolve 2 g of sodium hydroxide pellets in water using a 1 000 ml flask (5.2), add 2 g of sodium borohydride and dilute to the mark with water. Prepare a fresh solution daily and, when necessary, filter before use. When the analysis procedure requires a longer time it is recommended to cool the sodium borohydride solution, i.e. with ice around the flask, during its use in the CVAAS measurement.

NOTE 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 Mercury stock solution, $c(\text{Hg}) = 1\ 000$ mg/l.

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

Otherwise, dissolve 1,080 g of mercury(II) oxide in 10 ml of potassium dichromate solution and dilute to 1 l with water. Use a potassium dichromate solution with a concentration of 5 g/l. Dissolve 5 g of potassium dichromate with 500 ml nitric acid (4.1) and dilute to 1l with water.

4.7 Mercury standard solution, $c(\text{Hg}) = 1$ mg/l.

Dilute 100 μl the stock solution (4.6) with diluted hydrochloric acid (4.4) in a 100 ml flask (5.2) to a concentration of 1 mg/l.

The standard solution is stable for at least three months.

4.8 Mercury calibration solutions.

Dilute the standard solution (4.7) to the concentrations needed for calibration with diluted hydrochloric acid (4.4).

The following calibration solutions are recommended (see Table 1). Take aliquots of 0 μl , 25 μl , 50 μl , 250 μl , 500 μl of the mercury standard solution (4.7) into flasks of 50 ml (5.2) and fill up with diluted hydrochloric acid (4.4) to concentrations of 0 $\mu\text{g/l}$, 0,5 $\mu\text{g/l}$, 1 $\mu\text{g/l}$, 5 $\mu\text{g/l}$ and 10 $\mu\text{g/l}$.

Table 1 — Recommended calibration solutions (4.8) for the determination of mercury

Mercury (Hg)	Concentration of calibration solution (4.8)	Aliquots of standard solution (4.7), transferred in 50 ml flasks
	$\mu\text{g/l}$	μl
Calibration standard 1	0	0
Calibration standard 2	0,5	25
Calibration standard 3	1	50
Calibration standard 4	5	250
Calibration standard 5	10	500

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

5 Apparatus and equipment

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

NOTE Recommendations are given in EN 13804.

5.1 Microwave-heated pressure digestion apparatus with inert reaction vessels, made of materials such as Polytetrafluorethen (PTFE), Polyfluoralkan (PFA), Perfluorethylenpropylen (FEP) or quartz and which are suitable for digestion temperatures exceeding 200 °C.

The microwave oven should be generally resistant to corrosion. In particular, the whole electronic area of the microwave oven should be protected against corrosion to ensure safe operation. The ventilation should transfer the acid vapours to an extractor hood.

The reaction vessels should have a safety valve designed for a pressure of 10 000 kPa.

5.2 Pipettes, Flasks, of the following capacities: 25 ml, 50 ml, 100 ml, 500 ml and 1 000 ml.

5.3 Flow injection cold-vapour system with sample loop, i.e. 500 μl .

5.4 Atomic absorption spectrometer (AAS), with a heated quartz cell and optionally with an amalgamation system.

5.5 Element-specific lamp for mercury.

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

5.6 Ultrasonic bath or water bath.

5.7 Analytical balance, accurate to 0,1 mg.

6 Procedure

6.1 General

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

To ensure homogeneity, the use of a stationary or, especially for mineral feeds, a rotary riffler for mass reduction and the use of a sieve size of 0,5 mm or lower for particle size reduction are recommended because of the low weights of $\leq 0,5$ g of the test portions.

6.2 Preparation of the test solution

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

The mass 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 $\leq 0,5$ mm) test sample is weighed to an accuracy of 1 mg for digestion.

Add for example 5 ml nitric acid (4.1) and 2,5 ml hydrogen peroxide (4.2) using reaction vessels of 100 ml size. Ensure that the reaction vessels are locked and fixed in the microwave digestion system (5.1).

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 for a sufficient period of time, 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, taking into consideration the operating manual of the manufacturer.

WARNING 2 — For samples of an unknown composition, firstly carry out a digestion procedure with a small test portion. In particular cases, heavy reactions with nitric acid and/or hydrogen peroxide could appear. Formation of highly explosive compounds is also possible when organic matrices are digested. Too high test sample masses could result in uncontrollable reactions.

In principle, the pressure digestion is started with low power which is 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 2 With a digestion temperature of 200 °C, a sufficient extraction of mercury (and other elements) is obtained. In general, it is the case that the quality of the digestion will become better with increasing digestion temperature.

Remove the reaction vessels from the microwave system in an extractor hood and release the pressure carefully before opening. Let the vessels stand open 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 (5.6) is recommended to degas the extraction solution.

When reaction gases are being lost during microwave digestion, the whole extraction procedure should be repeated with a reduced test portion. This loss becomes very obvious when the volume of the extraction solution is reduced after the pressure digestion procedure.