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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)

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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 (SAVVF) après digestion sous pression par micro-ondes (extraction avec de l'acide nitrique à 65 % et du peroxyde d'hydrogène à 30 %)

Futtermittel - Bestimmung von Quecksilber mit Kaltdampf-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of an identical text or by endorsement, at the latest by January 2013, and conflicting national standards shall be withdrawn at the latest by January 2013.

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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 (HNO_3), not less than 65 % (mass fraction), of approximately $\rho(HNO_3)$ = 1,4 g/ml.

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

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

4.3 Hydrochloric acid (HCl), not less than or equal to 30 % (mass fraction), of approximately $\rho(HCl) \geq 1{,}15$ 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, $c(SnCl_2 \cdot 2H_2O) = 100$ 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. $c(NaBH_4) = 2$ 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(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(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 µg/l, 0,5 µg/l, 1 µg/l, 5 µg/l and 10 µg/l.

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

Mercury (Hg)	Concentration of calibration solution (4.8) µg/l	Aliquots of standard solution (4.7), transferred in 50 ml flasks 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

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 shich 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 ≤ 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 $\underline{<}$ 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 de-gas 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.

The vessels should be weighed before and after the microwave digestion procedure. When the difference in weight is more than 10 % of the volume of the used chemical agents (HNO_3 and H_2O_2), the extraction procedure should be repeated. In such cases,,loss of the analyte is very likely.

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

The extraction solution should be clear. When there are suspended particles in the extraction solution, let them settle to the bottom of the flask or either filter or centrifuge the solution before transferring it to a vessel of PP, PFA or FEP. If the measuring is not done immediately after the extraction, the test solution shall be stored in adequate (quartz) vessels to prevent a loss or a carryover of mercury.

6.3 Measurement of the test solution

6.3.1 Spectrometer settings of the cold-vapour atomic absorption spectrometer (CVAAS)

To devise a test schedule, firstly 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 tin(II) chloride or sodium borohydride introduced. Typical settings are listed in Table 2.

Table 2 — Typical settings of CVAAS for measuring mercury

Temperature of the cell	100 °C
Wave length	253,7 nm
Slit width	0,7 nm
Signal processing [a]	Signal height
Smoothing	0,5 s
Integration time	15 s

[a] Nearest to the limit of quantification a signal processing by signal area is recommended.

6.3.2 CVAAS determination

The test and calibration solutions are measured directly with an atomic absorption spectrometer and with an electrically heated quartz cell coupled to a flow injection cold-vapour 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 test or 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 (4.5.2) or with tin(II) chloride solution (4.5.1). The resulting gas/liquid mixture is separated by an argon-flow separator. The argon steam sorts out the elemental volatile mercury to the quartz cell for measuring the atomic absorption of mercury.

Optionally, an amalgamation system could be used to increase sensitivity and to decrease matrix effects.

The calibration solutions are measured first; then the test solutions are measured.

Check the linear range of the calibration function. If the concentration of the test sample is outside the linear range, dilute it with diluted 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 regular intervals.

Although a correction is seldom necessary in the case of the cold-vapour technique, whether the background correction is necessary or not shall be checked for every type of sample. As an analytical control, reference samples having reliable known mercury contents shall be analysed parallel with 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 the steps in the method shall also be determined.

NOTE Other elements in high concentration levels like selenium, iodide and copper extracted from the test sample matrix (for example from pre-mixtures) can interfere and lead to lower measured mercury values of the test solution. In cases where there are complex or unknown matrices, the standard addition method can be used. More detailed information about the tolerable concentration levels of interfering elements in the test solution (mostly in the range of in maximum 0,5 mg/l to 1 mg/l) is given in Table 1 of EN 1483:2007, 4.2.

7 Calculation

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

The mercury mass fraction of the weighed test sample (w_s) is calculated according to following formula:

$$W_S = (c_t - c_b) \times V \times D / (m \times 1000) \ (mg/kg) \quad (1)$$

where

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

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

m is the mass of test portion, in g;

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

D is the dilution factor.

EXAMPLE:

Using m = 0,5 g as the test portion, a graduated 25 ml flask (5.2) for microwave digestion (V = 25 ml) when no further dilution (D = 1) is performed, the mercury mass fraction of the sample (= W_S) is calculated as:

$$W_S = (c_t - c_b) \times (25 \times 1) / (0{,}5 \times 1\,000) = (c_t - c_b) \times 25/500 = (c_t - c_b)/20 \quad (mg/kg)$$

8 Precision

8.1 Introduction

An interlaboratory comparison was organized by the Technische Universität München, Research Center for Nutrition and Food Sciences, Bioanalytic Weihenstephan in 2010. The results of the main method protocol using pressure digestion and CVAAS determination are given in Annex A. Results of other extraction procedures and/or other systems like Direct Mercury Analyser (DMA) which were used as alternatives in this interlaboratory study were compliant with those of the main protocol. Details are given only in the final report [17].

8.2 General

Details of an interlaboratory test on the precision of the method are summarised in Annex A. The values derived from this interlaboratory 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 not more than 5 % of the cases exceed the values of r (repeatability limit) given in Table 3.

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 not more than 5 % of the cases exceed the values of R (reproducibility limit) given in Table 3.

Table 3 — Precision data

Matrix	Mean mg/kg	Repeatability limit r mg/kg	Reproducibility limit R mg/kg
Mineral piglet feed	0,005	0,003	0,005
Fish meal	0,087	0,008	0,035
Fish feed	0,174	0,015	0,060
Rabbit feed	0,754	0,095	0,237
Dicalciumphosphate	2,003	0,237	0,410
Brewers grains	0,464	0,066	0,154

9 Test report

The test report shall specify:

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 which might have influenced the test result(s).

Annex A
(informative)

Results of the interlaboratory tests

Table A.1 — Precision data

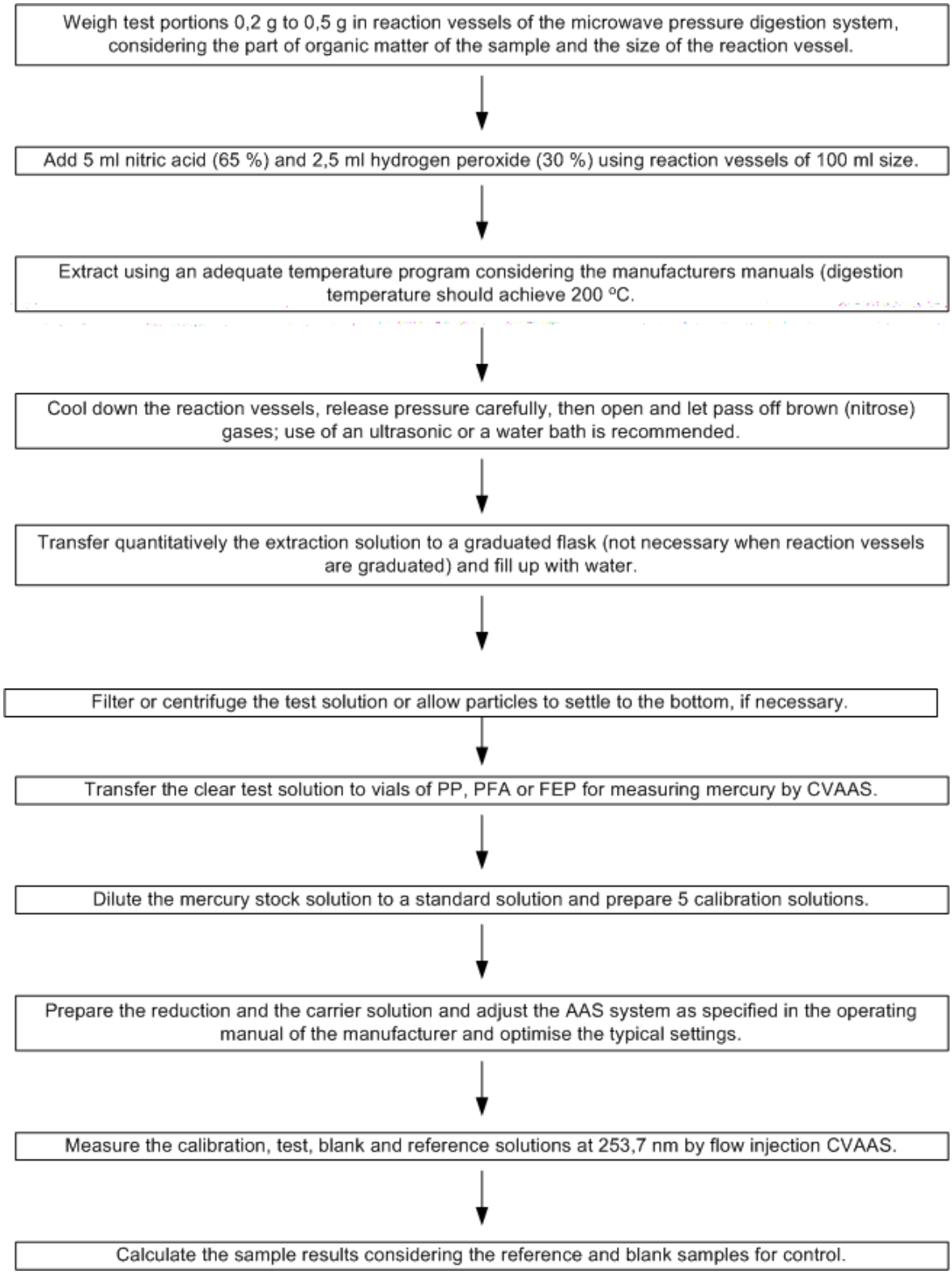
Matrix tested	Mineral piglet feed	Fish meal	Fish feed	Rabbit feed	Dicalcium phosphate	Brewers grains	Fish feed	Mineral piglet feed
Subsamples fully prepared for direct weighing	yes	yes	yes	yes	yes	yes	no	no
Number of labs	20	20	20	20	20	20	20	20
Number of outlier labs	3	2	2	3	3	3	2	4
Number of labs without outliers	17	18	18	17	17	17	18	16
Mean value, mg/kg	0,005	0,087	0,174	0,754	2,003	0,464	0,092	0,006
Repeatability standard deviation $s_{r,}$ mg/kg	0,001	0,003	0,005	0,034	0,085	0,024	0,004	0,001
Repeatability limit r, mg/kg	0,003	0,008	0,015	0,095	0,237	0,066	0,010	0,002
Coefficient of variation of Repeatability, CV_r, %	17,6	3,4	3,1	4,5	4,2	5,1	3,8	12,3
Reproducibility standard deviation $s_{R,}$ mg/kg	0,002	0,013	0,021	0,085	0,146	0,055	0,011	0,002
Reproducibility limit R, mg/kg	0,005	0,035	0,060	0,237	0,410	0,154	0,032	0,007
Coefficient of variation of Reproducibility, CV_R, %	31,6	14,3	12,3	11,2	7,3	11,9	12,5	37,8
HORRATvalue (R)[b]	1,4 [a]	0,7 [a]	0,6	0,7	0,5	0,7	0,6 [a]	1,7 [a]

[a] Thompson (2000) [16].

[b] The HORRAT value is the Horwitz –Ratio value.

Annex B
(informative)

Flowchart – Determination of mercury by CVAAS after microwave digestion



Annex C
(informative)

Alternative digestion procedure with the same extraction efficiency: Acid digestion with a mixture of 65 % nitric acid and 70 % perchloric acid (7:3 v/v) at atmospheric pressure

C.1 General

WARNING — Work with perchloric acid should only be undertaken if safety precautions are followed and care, caution, chemical knowledge and common sense are used. It should be pointed out that the safety depends not only on rules (see C.5), time- and temperature-controlled automated decomposition, special hood, exhaust system and sprinkler-washing system, but also on conscientious co-workers with a sense of responsibility.

C.2 Preparation of the test solution

C.2.1 Introduction

Weigh 1 g dry or 5 g wet test portion in a sample tube (80 ml). Add 15 ml of a mixture of 65 % nitric acid and 70 % perchloric acid (7:3 v/v) of ultrapure quality. Let the solution stand for 1 h at room temperature.

Automatic digestion of the test solution is performed overnight using an electrically heated block of aluminium connected to a microprocessor for control of temperature and time. Dilute the test solution to 25 ml or 50 ml with 0,5 mol/l hydrochloric acid.

C.2.2 Digestion program

Step	Temperature °C	Ramp h	Time h
1	30	0:15	1:45
2	50	0:15	1:45
3	70	0:15	1:45
4	100	0:15	1:45
5	120	0:15	1:45
6	138	1:15	1:00
7	150	1:30	1:30
8	160	0:45	0:45
9	180	0:45	0:45

C.3 Mercury calibration solutions

Add 0 ml, 0,1 ml, 0,4 ml, 0,8 ml, 1,2 ml and 1,6 ml of a 1 000 µg/l mercury standard solution into 100 volumetric flasks. Add 0,5 mol/l hydrochloric acid to the mark. The concentrations of the calibration solutions are: 0 µg/l, 1 µg/l, 4 µg/l, 8 µg/l, 12 µg/l and 16 µg/l.

C.4 Measurement of the test solution: Atomic absorption spectrometer (CVAAS-procedure)

The test solution is measured using CVAAS as described in section 6.3.

The test solution can also be determined by using FI-CVAAS or CV-ICP-AES according to user's equipment and the recommendations of the manufacturer.

C.5 Ten rules for automated wet ashing with perchloric acid

1) Always elaborate a new temperature program and a suitable mixture of the oxidizing acids for material with unknown chemical properties. The development shall be tested both stepwise and by visual control.

2) A maximum of 5 g material wet weight (1 g dry mass) containing no more than 500 mg fat is allowed when using 15 ml oxidizing acid mixture (HNO_3/$HClO_4$: 7/3 v/v).

3) Add oxidizing acids to the samples. Digest always in the form of a mixture, never separately.

4) Mark the meniscus on the tube for the control of the decreasing acid mixture during the ashing procedure.

5) Digest the samples at ambient temperature for 3 h to 5 h before starting the ashing program.

6) Prevent bumping of acid solution at boiling. Such movement is disastrous for the analysis.

7) Solubilize fat and fatty acids at 132 °C until homogeneity of phases is achieved.

8) Dark colour during digestion indicates danger. If dark colour appears, remove the tubes from the block and repeat the digestion after an addition of HNO_3. Mercury can be lost by charring.

9) Digest at 180 °C over night. Digest according to the program, only tubes with light coloured solutions.

10) Hoods are made from polypropylene, exhaust tubes and fan of PVC. Therefore, wash the whole system regularly from hood to the fan with water.

Annex D
(informative)

Alternative digestion procedure with the same extraction efficiency: Acid digestion with a mixture of 65 % nitric acid, 37 % hydrochloric acid and 30 % hydrogen peroxide under reflux

D.1 Preparation of the test solution

Weigh 2 g of the test portion in a 50 ml flask and add 10 ml of 65 % nitric acid (4.1) and 7,5 ml of 37 % hydrochloric acid (4.3). Let the solution stand for 1 h at room temperature. Add 4 ml of 30 % hydrogen peroxide (4.2). 2 drops of octanol can be added to avoid heavy foaming.

Heat for 30 min at 60 °C and for 120 min at 120 °C under reflux.

Dilute the test solution to 100,0 ml with diluted hydrochloric acid (4.4). The acid concentration of the test solution is approximately 3 % hydrochloric acid and 6 % nitric acid after dilution.

Filter the test solution and then store it in a cold dark area.

D.2 Mercury calibration solutions

Mercury calibration solutions are prepared according to 4.8.

Add 5 ml concentrated nitric acid (4.1) to each 50 ml flask before filling the flask with hydrochloric acid to match the acid content of the test solutions.

D.3 Measurement of the test solution

The test solution is measured with CVAAS, as described in 6.3.

Bibliography

[1] EN 13804:2002, *Foodstuffs — Determination of trace elements — Performance criteria, general considerations and sample preparation*

[2] EN 13805:2002, *Foodstuffs — Determination of trace elements — Pressure digestion*

[3] EN 13806:2002, *Foodstuffs — Determination of trace elements — Determination of mercury by cold-vapour atomic absorption spectrometry (CVAAS) after pressure digestion*

[4] EN 1483:2007, *Water quality — Determination of mercury — Method using atomic absorption spectrometry*

[5] EN 15550:2007, *Animal feeding stuffs — Determination of cadmium and lead by graphite furnace atomic absorption spectrometry (GF-AAS) after pressure digestion*

[6] EN 15621:2012, *Animal feeding stuffs — Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES*

[7] VDLUFA, chapter VII, 2.1.3 (2007) — *Microwave heated pressure digestion procedure*

[8] VDLUFA, chapter VII, 2.2.2.9 (2008) — *Determination of mercury in feeding stuffs, cold-vapour-AAS-method*

[9] Frank A., (1976), *Automated wet ashing and multi-metal determination in biological materials by atomic absorption spectrometry*. Z. Anal. Chem., 279, pp. 101-102

[10] Frank A., (1988), 'Semi-micro accessory to an automated digestion system for ashing small sample amounts', in Brätter P. and Schramel P. (Eds.) *Trace Element Analysis in Medicine and Biology*, Vol. 5, Walter de Gruyter & Co., Berlin, pp. 78-83

[11] Galgan V. and Frank A., (1988), 'Automated system for determination of selenium in biological materials', in: Brätter P., Schramel P. (eds.), *Trace elements Analytical Chemistry in Medicine and Biology*, Vol.5, Walter de Gruyter & Co., Berlin, New York, pp. 84-89

[12] Galgan V. and Frank A., (1993), 'Notes and comments on the determination of selenium in biological materials', in *Norwegian Journal of Agricultural Sciences*, Supplement No. 11, pp. 57-74

[13] Kaiser G., Götz D., Tölg G., Knapp G., Maichin B., Spitzy H., (1978), 'Untersuchung von systematischen Fehlern bei der Bestimmung von Hg-gesamt-gehalten im Bereich $< 10^{-5}$ % in anorganischen und organischen Matrices mit zwei unabhängigen Verbundverfahren', in Fresenius Z. *Anal. Chem*. 291, pp. 278 - 291

[14] Kruse, R., (1979), 'Ein verlustfreier offener Aufschluss mit HNO_3 / $HCLO_3$ / $HCLO_4$ für die Bestimmung von Gesamt-Quecksilber in Fischen', in Z. Lebensm. Unters. Forsch. 169, pp. 259 - 262

[15] Schaller K.H., (1988), 'Mercury Determination in blood, and urine. Atomic absorption spectrometry - cold-vapour method', in Angerer J., Schaller K.H. (eds.), *Analysis in Biological Materials*, Vol. 2, Verlag Chemie (VCH), Weinheim, Germany, pp. 195 – 211

[16] Thompson M., (2000), 'Recent trends in interlaboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing' in *Analyst*, 125, pp. 385 - 386.

[17] Validation of an analytical method to determine mercury (Hg) in animal feeding stuffs - Final report of the collaborative study - Animal feeding stuffs – Determination of mercury by cold-vapour atomic absorption spectrometry (CVAAS) after microwave digestion (extraction with 65 % nitric acid and 30 % hydrogen peroxide), Jürgen Danier (Project leader) c/o Bioanalytik Weihenstephan, Research Centre for Nutrition and Food Science (ZIEL) of TUM, 85350 Freising, Germany: http://www.wzw-bioanalytik.de/download_e.php

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