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Animal feeding stuffs — Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave 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 selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65 % nitric acid and 30 % hydrogen peroxide)

Aliments pour animaux - Dosage du sélénium par spectrométrie d'absorption atomique par génération d'hydrures (SAAGH) après digestion par micro-ondes (extraction avec de l'acide nitrique à 65 % et du peroxyde d'hydrogène à 30 %)

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

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Foreword

This document (EN 16159:2012) 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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.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 selenium in animal feeding stuffs by hydride generation atomic absorption spectrometry (HGAAS) after microwave pressure digestion.

The method was successfully tested by an inter-laboratory study of CEN/TC 327/WG 4 in the range of 0,25 mg/kg to 74 mg/kg.

The limit of quantification is 0,5 µg/l of the test solution which corresponds to the calibration standard 2. Using a test portion of 0,5 g and a volume of the test solution of 25 ml after pressure digestion the limit of quantification is calculated as 0,125 mg/kg in the feed material.

NOTE A lower limit of quantification could be achieved – each laboratory has to prove it.

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

Selenium is determined in the test solution by hydride generation atomic absorption spectrometry (fluorescence hydride generation atomic absorption) 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.

Selenium ions of the test solution are reduced with hydrochloric acid to selenium (IV) and converted to selenium hydride (SeH_2) by sodium borohydride. This selenium hydride is transferred by a gas stream to a heated measurement cell and decomposed. The absorption at the selenium line at 196,0 nm corresponds to the amount of selenium.

NOTE Selenium (VI) is not determined by the hydridisation as described here. It is therefore necessary to adjust the digestion conditions and to exercise a pre-reduction step with hydrochloric acid to yield only selenium (IV).

Other digestion procedures with the same digestion efficiency or other measurement systems like FI-HGAAS or hydride generation inductively coupled plasma optical emission spectrometry are possible (see Annex C).

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 selenium in the reagents and apparatus used.

Use only water of quality 2 as described in EN ISO 3696.

NOTE High purity is essential to avoid potential contamination. Therefore, only use reagents available with high purity or perform an extraction 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 Diluted nitric acid, mix 100 ml nitric acid (4.1) with water to 1 l.

4.3 Hydrogen peroxide, not less than 30 % (mass fraction), of approximately ρ (H_2O_2) ≥ 1,1 g/ml.

4.4 Hydrochloric acid, 30 %, mass concentration of approximately ρ (HCl) = 1,15 g/ml.

4.5 Diluted hydrochloric acid, e.g. about 3 % (mass fraction), as carrier solution for the use in the flow-injection-procedure.

EXAMPLE Dilute approximately 90 ml of hydrochloric acid (4.4) to 1 l with water.

4.6 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.

4.7 Selenium stock solution, c (Se) = 1 000 mg/l.

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

Otherwise, dissolve 1,4053 g of selenium dioxide (SeO_2) and 2 g sodium hydroxide in approximately 50 ml water, and dilute to 1 000 ml with water.

4.8 Selenium standard solution, c (Se) = 1 mg/l.

Dilute e.g. 100 µl of the stock solution (4.7) in a 100 ml flask (5.3) to give a concentration of 1 mg/l. The selenium standard solution shall contain an adequate amount of hydrochloric acid, e.g. 2 ml of hydrochloric acid (4.4) per 100 ml.

NOTE The standard solution is stable for at least three months.

4.9 Selenium calibration solutions.

For the preparation of five calibration solutions, the following procedure is recommended: Take aliquots of 0 µl, 50 µl, 250 µl, 500 µl and 1 000 µl of the selenium standard solution (4.8) into 100 ml flasks (5.3). After the addition of 20 ml of nitric acid (4.2) and 10 ml of hydrochloric acid (4.4) the calibration solutions are heated for 20 min in a water bath at 80 °C (see pre-reduction step 6.3.2). After cooling down to room temperature, the flasks (5.3) are made up to the mark with water and the calibration solutions are measured.

The selenium concentrations of the calibration solutions are: 0 µg/l; 0,5 µg/l; 2,5 µg/l; 5 µg/l and 10 µg/l (see Table 1).

Table 1 — Recommended calibration solutions (4.9) for the determination of selenium

Selenium (Se)	**Concentration of calibration solution (4.9) after pre-reduction procedure** µg/l	**Aliquot of selenium standard solution (4.8) transferred in 100 ml flasks (5.3) (pre-reduction step)** µl
Calibration standard 1	0	0
Calibration standard 2	0,5	50
Calibration standard 3	2,5	250
Calibration standard 4	5	500
Calibration standard 5	10	1 000

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 especially the electronics should be 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, 100 µl, 250 µl, 600 µl, 1 000 µl, 1 500 µl, 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 Element-specific lamp for selenium.

NOTE An electrodeless discharge lamp would provide a higher sensitivity compared 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 selenium and for other minerals and trace elements which correspond to the total contents of these elements. For some specific problems 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 exactly 1 mg for digestion.

Add e.g. 5 ml nitric acid (4.1) and 2,5 ml hydrogen peroxide (4.3) 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 addition of nitric acid and hydrogen peroxide. Therefore, let the reactions fade off at room temperature, 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 considering the operating manual of the manufacturer.

WARNING 2 — For samples with unknown composition a digestion procedure with low test portions should be performed at first. 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. The system is then cooled down.

NOTE 3 With a digestion temperature of 200 °C a sufficient digestion of selenium (and other elements) is obtained. In general, it applies that the quality of the digestion will become better with increasing digestion temperature. Digestion temperatures greater than 280 °C can lead to increased formation of volatile selenium (VI).

Remove the reaction vessels from the microwave system in an extractor hood or a fume cupboard and release the pressure carefully before opening. 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 reaction gases are lost during the microwave digestion the whole digestion procedure should 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 or 50 ml flask (5.3) and filled up to the mark with water. For graduated reaction vessels, the digestion solution could be directly 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 down, filtrate 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 must be stored in suitable vessels to prevent a loss or a carryover of selenium.

6.3 Measurement of the test solution

6.3.1 Pre-dilution of the test solution

It is very important that the acid concentration of the (diluted) test solution corresponds to that of the calibration solutions because the signal height for measuring selenium by HGAAS depends on the acid matrix.

Therefore, when the measured selenium concentration of a sample exceeds the linear range of the calibration function, a dilution of the test solution (6.2) with nitric acid (4.2) instead with water is necessary.

EXAMPLE With a (linear) calibration function of calibration solutions of 0,5 µg/l to 10 µg/l feeding stuff samples with selenium concentrations of 0,125 mg/kg to 2,5 mg/kg can be measured without further dilution using 0,5 g test portion, a 25 ml flask (5.3) after microwave pressure digestion, an aliquot of 10 ml of the digestion solution for pre-reduction using a 50 ml flask (5.3). Thus samples with higher selenium concentrations should be diluted in such a way that the selenium concentration of the pre-reduced test solution falls within the calibration function.

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

Transfer 10 ml of the test solution (6.2) or the pre-diluted test solution (6.3.1) to a 50 ml flask (5.3), add 5 ml hydrochloric acid (4.4) and heat for 20 min in a water bath at 80 °C with opened flask. After cooling down to room temperature, the flask is made up to the mark with water.

6.3.3 Spectrometer settings of the flow-injection hydride generation atomic absorption spectrometer (HGAAS)

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 2.

Table 2 — Typical settings of HGAAS for measuring selenium

Temperature of the cell	**900 °C**
Wave length	**196,0 nm**
Slit width	**2,0 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 (6.3.2), if necessary pre-diluted previously (6.3.1), and the selenium 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 (4.5) and mixed with sodium borohydride solution (4.6). The resulting gas/liquid mixture is separated by an argon-flowed separator. The argon steam separates and transports the metal hydrides to the quartz cell for atomisation reaction and measurement of the atomic absorption of selenium.

Firstly, the selenium calibration solutions (4.9) are measured, then the (pre-diluted) test solutions (6.3.2).

Check the linear range of the calibration function. If the concentration of the test sample is outside the linear range, dilute with nitric acid (4.2) and not with pure water. When carrying out prolonged series of measurements, it is advisable to check the zero and the calibration at intervals.

Significant background signal appears in the case of the hydride generation technique, either by matrix effects or by using higher concentrations of nitric acid or hydrochloric acid. Add amido sulphuric acid after the pre-reduction step when disturbances from nitric acid appear.

Copper concentrations in the test solution of more than 750 µg/l could lead to a signal depression. Measuring copper and selenium concentrations simultaneously by ICP-AES could be useful: If the selenium concentration of the test solution by ICP-AES is higher to that of HGAAS and if the copper concentration is high, a depression effect by copper seems to be possible. Then dilute the test solution (6.3.1) with diluted nitric acid (4.2) or take 1 ml of a 0,5 % solution of 1,10 phenanthroline into 10 ml of the test solution (= 0,05 % of 1,10 phenanthroline within the test solution) for complexation of copper to measure selenium by HGAAS without a signal depression.

For unknown matrix effects use the standard addition procedure.

As an analytical control, reference samples having reliable known selenium 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.

7 Calculation

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

The selenium 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 selenium in the test solution, µg/l;

c_b is the concentration of selenium 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. 10), ml;

V_3 is the volume for pre-reduction step (i.e. 50), ml;

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

1 000 is the constant factor to calculate from selenium 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 10 ml after digestion (= V_2) and a 50 ml flask (5.3) for the pre-reduction step (= V_3) - the selenium mass of the sample (= W_s) is calculated as mg/kg in the feed material when no further dilution (D = 1) is done:

$$W_S = (c_t - c_b) \times (25 \times 50 \times 1) / (0{,}5 \times 10 \times 1\ 000) = (c_t - c_b) \times 1\ \ 250 / 5\ \ 000 = (c_t - c_b) / 4 \quad mg/kg$$

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. The results of the main method protocol using pressure digestion and HGAAS determination are given in Annex A. Results of other digestion procedures and/or other systems like inductively-coupled-plasma mass-spectrometry 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 [14].

8.2 General

Details of an inter-laboratory test done in 2009 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 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 no more than 5 % of the cases, exceed the values of R given in Table 3.

Table 3 — Precision data

Matrix	mean, mg/kg	r, mg/kg	R, mg/kg
Cow feed	0,305	0,072	0,192
Turkey feed	0,313	0,054	0,134
Wheat	0,049	0,050	0,080
Mineral piglet feed	6,87	1,94	4,23
Mineral cow feed	73,6	6,9	34,5

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 Wheat contains low selenium content in the range of the limit of quantification with a HORRAT(R) of 2,3.

Matrix tested	Cow feed	Turkey feed	Wheat	Mineral pig feed	Mineral cow feed	Turkey feed	Mineral pig feed
Subsamples fully prepared for direct weighing	yes	yes	yes	yes	yes	no	no
No. of labs	19	19	19	19	19	18	18
No. of outlier labs	1	4	3	0	4	4	1
No. of non-compliant labs	0	0	0	3	2	0	2
No. of valid labs	18	15	16	16	13	14	15
Mean value, mg/kg	0,305	0,313	0,049	6,87	73,6	0,246	6,76
s_r, mg/kg	0,026	0,019	0,018	0,69	2,5	0,016	0,58
r, mg/kg	0,072	0,054	0,050	1,94	6,9	0,045	1,62
RSD(r), %	8,5	6,1	36,3	10,1	3,4	6,5	8,6
$S_{R,}$ mg/kg	0,069	0,048	0,027	1,51	12,3	0,037	1,57
R, mg/kg	0,192	0,134	0,080	4,23	34,5	0,103	4,39
RSD(R), %	22,5	15,3	58,3	22,0	16,7	14,9	23,2
HORRAT(R)	1,2	0,8	2,3	1,8	2,0	0,8	1,9

Annex B
(informative)

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

Weigh test portions of 0,2 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 manufacturer's manuals (digestion temperature should achieve 200 °C).

↓

Cool down the reaction vessels, release pressure carefully, then open and allow brown (nitrose) gases to be released 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 selenium by HGAAS.

↓

Dilute the selenium stock solution to a standard solution. Prepare at the day of analysis 5 pre-reduced calibration solutions with hydrochloric acid (30 %) in a water bath for 20 min at 80 °C.

↓

If necessary pre-dilute the test solutions with diluted nitric acid, then pre-reduce the test solutions with hydrochloric acid (30 %) in a water bath for 20 min at 80 °C.

↓

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 196,0 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: Acid digestion with a mixture of 65 % nitric acid and 70 % perchloric acid (7:3 by volume) at atmospheric pressure

NOTE 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.4), 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.1 Preparation of selenium calibration solutions

Add 0 ml, 0,2 ml, 0,5 ml, 1,0 ml, 1,5 ml and 2,0 ml of a 1 000 µg/l selenium standard solution into 100 ml flasks. Add 5 mol/l hydrochloric acid (not to mark) and pre-reduce Se (VI) to Se (IV) by heating the calibration solutions for 30 min at 100 ˚C. After cooling, dilute the solutions to the mark of the flask. The final concentrations of the calibration solutions are:

0 µg/l, 2 µg/l, 5 µg/l, 10 µg/l, 15 µg/l and 20 µg/l.

C.2 Preparation of the test solution

Weigh 1 g dry or 5 g wet test portion in a sample tube (80 ml), i.e. a Kjeldahl tube. Add 15 ml of a mixture of 65 % nitric acid and 70 % perchloric acid (7:3 by volume), the reagents should be of ultrapure quality. Immediately after addition of the acid mixture, the digest is initiated. Automatic digestion of the test solution is performed using an electrically heated block of aluminium connected to a microprocessor for control of temperature and time according to a standard digestion program [11][12][13][14].

Table C.1 — Temperature-time program for digestion

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	132	1:15	1:00
7	150	1:30	1:30
8	160	0:45	0:45
9	180	0:45	1:00
10	225	0:30	1:00

C.3 Measurement of the test solution

C.3.1 Pre-reduction of Se (VI) to Se (IV) of samples

Add 10 ml 5 mol/l hydrochloric acid to the sample test tube. Heat the tube in the block for 30 min at 110 °C. After cooling add 5 mol/l hydrochloric acid to 25 ml (calibrated tubes).

C.3.2 Atomic absorption spectrometer (HGAAS-procedure)

The test solution is measured using HGAAS as described in 6.3.4.

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

C.4 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 has to be tested step-by-step and by visual control.

2) Max 5 g material wet wt (1 g DM) and containing no more than 500 mg fat are allowed when using 15 ml oxidizing acid mixture (HNO_3/$HClO_4$: 7/3 by volume fraction).

3) Add oxidizing acids to the samples to always digest in the form of a mixture. Never separately!

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

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

6) Prevent bumping of acid solution at boiling. It is disastrous for the analysis!

7) Solubilise fat and fatty acids at 132 °C until homogeneity of phases.

8) Dark colour during digestion indicates danger. Remove the tubes from the block and repeat the digestion after addition of HNO_3. Selenium can be lost by charring.

9) Stop digestion temperature at 180 (± 5) °C after overnight digestion. Increase temperature to 225 (± 5) °C and digest according to the program,only tubes with light coloured solutions.

10) Hoods are made from PP (polypropylene), exhaust tubes and fans are made from PVC (polyvinyl chloride). Wash the whole system from hood to fan regularly with water.

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