BRITISH STANDARD

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Foodstuffs — Determination of trace elements — Determination of tin by flame and graphite furnace atomic absorption spectrometry (FAAS and GFAAS) after pressure digestion

ICS 67.050

National foreword

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Contents

Page

Foreword

This document (EN 15764:2009) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by June 2010, and conflicting national standards shall be withdrawn at the latest by June 2010.

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1 Scope

This European Standard specifies a method for the determination of tin in foodstuffs and canned foods by flame and graphite furnace atomic absorption spectrometry (AAS) after pressurized digestion.

The collaborative study included foodstuffs such as carrot puree, tomato puree, pineapple, mixed fruit, white wine, peach powder, tomato powder, powdered beans, powdered fruit yoghurt, fish powder, having mass fractions of tin ranging from 43 mg/kg to 260 mg/kg (Flame-AAS) and from 2,5 mg/kg to 269 mg/kg (Graphite Furnace AAS).

2 Normative references

The following referenced documents are indispensable for the application 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 13805, *Foodstuffs — Determination of trace elements — Pressure digestion*

3 Principle

The sample is mineralized through pressurized digestion with nitric acid and hydrochloric acid in accordance with EN 13805. In the resulting digestion solution, tin is quantified by flame AAS (F-AAS) or graphite furnace AAS (GF-AAS) depending on the concentration in the sample solution.

4 Reagents

4.1 General

The concentration of tin in the reagents and water used shall be low enough not to affect the results of the determination.

4.2 Nitric acid

Mass fraction $w(HNO_3) \geq 65$ %, mass concentration $\rho(HNO_3) \approx 1{,}4$ g/ml.

4.3 Hydrochloric acid

$w(HCL) \geq 30$ %, $\rho(HCl) \approx 1{,}15$ g/ml.

4.4 Tin stock solution

$\rho(Sn) = 1\,000$ mg/l.

4.5 Tin standard and calibration solutions

4.5.1 General

The standard and calibration solutions are prepared from the stock solution by dilution in glass volumetric flasks. For calibration, prepare at least four calibration solutions of different concentrations. The acid concentration shall correspond to the concentration in the measurement solution.

The preparation of the solutions in 4.5.2 and 4.5.3 is given as an example.

4.5.2 Calibration solutions

of ρ(Sn) = 5 mg/l, 10 mg/l, 20 mg/l and 30 mg/l for flame AAS.

Fill four 50 ml volumetric flasks with 10 ml to 20 ml of water, add 5 ml of nitric acid (4.2) and 1 ml of hydrochloric acid (4.3) and mix. Cool the solutions to ambient temperature, and pipette exactly 0,25 ml, 0,50 ml, 1,00 ml and 1,50 ml of tin stock solution (4.4) for the respective calibration solutions of mass concentrations 5 mg/l, 10 mg/l, 20 mg/l and 30 mg/l into the four different 50 ml volumetric flasks. Mix the solutions and dilute to volume with water. These solutions are stable for at least one day.

The calibration solutions described here shall be understood as examples. The concentrations prepared shall be in the linear range of the measuring device. The acid concentration of the calibration solutions shall be matched to the acid concentration in the sample solution.

4.5.3 Calibration solutions

of ρ(Sn) = 0,010 mg/l, 0,020 mg/l, 0,040 mg/l and 0,060 mg/l for GF-AAS.

To prepare standard solution 1 (ρ(Sn) = 50 mg/l), fill a 50 ml volumetric flask with 10 ml to 20 ml of water, add 2,5 ml of hydrochloric acid (4.3) and mix. Cool the solution to ambient temperature, add exactly 2,5 ml of tin stock solution (4.4) and dilute to volume. This solution is stable for at least one week.

To prepare standard solution 2 (ρ(Sn) = 1,0 mg/l), fill a 50 ml volumetric flask with 10 ml to 20 ml of water, add 2,5 ml of hydrochloric acid (4.3) and mix. Cool the solution to ambient temperature, add exactly 1,0 ml of tin standard solution 1 by pipette and dilute to volume.

Prepare the calibration solutions for the graphite furnace AAS from standard solution 2 according to the following procedure:

Fill four 50 ml volumetric flasks with 10 ml to 20 ml of water, add 5 ml of nitric acid (4.2) and 1 ml of hydrochloric acid (4.3) and mix. Cool the solutions to ambient temperature, pipette exactly 0,50 ml, 1,0 ml, 2,0 ml and 3,0 ml of standard solution 2 for the respective calibration solutions of mass concentrations 0,010 mg/l, 0,020 mg/l, 0,040 mg/l and 0,060 mg/l into the four different 50 ml volumetric flasks and dilute to final volume with water. These solutions shall be re-prepared for each day of measurement.

The calibration solutions described here shall be understood as an example. The concentrations prepared shall be in the linear range of the measuring device. The acid concentration of the calibration solutions shall be matched to the amounts of acid used in the digestion.

4.6 Blank solution

The blank solution contains water, nitric acid and hydrochloric acid in amounts that correspond to the concentrations in the measurement solution, for example 10 ml of nitric acid (4.2) and 2 ml of hydrochloric acid (4.3) in 100 ml of water.

4.7 Matrix modifiers for graphite furnace AAS

4.7.1 General

Tin compounds shall be stabilized by matrix modifiers for quantification by graphite furnace AAS during the ashing step. Different matrix modifiers are used at different concentrations. To use a suitable modifier, first consider the recommendations of the device manufacturer. Select a suitable modifier; use the sample matrix to be examined to verify the ashing temperature of the graphite furnace program and optimize it in such a way that no tin is lost in the graphite tube during the mineralization step.

In 4.7.2 to 4.7.4 an example is presented of a modifier for quantifying tin.

4.7.2 Ammonium dihydrogen phosphate solution

Mass fraction $w \approx 10$ %.

Dissolve 10,0 g of ammonium dihydrogen phosphate ($NH_4H_2PO_4$) in 100 ml of water.

4.7.3 Magnesium nitrate solution

Mass concentration ρ(Mg) = 10 g/l.

Dissolve 10,5 g of magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$) in 100 ml of water.

NOTE Commercially available solutions can also be used.

4.7.4 Matrix modifiers for quantification of tin

$\rho(NH_4H_2PO_4)$ = 50 µg/10 µl and $\rho(Mg(NO_3)_2)$ = 3 µg/10 µl.

Pipette 2,5 ml of ammonium dihydrogen phosphate solution (4.7.2) and 0,25 ml of magnesium nitrate solution (4.7.3) into a 50 ml volumetric flask; add 1 ml of nitric acid (4.2), and dilute to volume with water.

This solution remains usable for several months when refrigerated.

5 Apparatus and equipment

5.1 General

All apparatus and equipment that come into direct contact with the sample and solutions shall be pre-cleaned appropriately.

5.2 Atomic absorption spectrometer

with nitrous oxide/acetylene burner and atomization system.

5.3 Atomic absorption spectrometer

with background correction (Zeemann background correction is recommended), graphite tube furnace and autosampler.

5.4 Element-selective detection

5.4.1 Element-specific lamp

for tin (hollow-cathode or electrodeless discharge lamp).

5.4.2 Continuum radiation source

with high-resolution monochromatic illuminator as an alternative to 5.4.1. The resolution of the measuring equipment at normal operation shall correspond to at least the half-width value of the emission line of the element specific lamp (usually 1 pm to 3 pm).

6 Procedure

6.1 Digestion of the sample

Mineralize the sample in pressurized digestion in accordance with EN 13805. For the quantification of tin, add 0,5 ml to 1 ml of hydrochloric acid (4.3) to the digestion vessel, which contains nitric acid (4.2) used for the digestion, at an amount that corresponds to the amount of nitric acid. Do not add the hydrochloric acid until the spontaneous reaction with nitric acid has subsided. After addition of the hydrochloric acid close the digestion vessel immediately in order to avoid loss of active chlorine. Start pressurized digestion shortly thereafter. The digestion requirements are based on the specifications of the instrument manufacturer, the reactivity of the sample, the maximum pressure stability of the digestion vessel and the attainable temperature.

EXAMPLE Precisely weigh 0,4 g to 0,5 g of dry sample (residual moisture of less than 20 %) in a 100 ml digestion vessel and mix it with 5 ml of nitric acid (4.2). Carefully shake the digestion vessel to prevent clots from forming in the sample. After the spontaneous reaction has subsided, add 1 ml of hydrochloric acid (4.3) and close the digestion vessel as quickly as possible. For samples with a higher degree of moisture content, the weighed quantities can be increased.

The digestion solution that results from the pressurized digestion according to EN 13805 can be used directly or can be diluted for the subsequent quantification of tin.

6.2 Flame atomic absorption spectrometry

6.2.1 General

Start the instrument and let it stabilise, then optimise it according to the manufacturer's specifications. Set the wavelength to 286,3 nm or 235,5 nm. The nitrous oxide/acetylene flame needs special attention. In general reducing conditions give the best response. If deposits are formed on the burner they need to be removed before they interfere with the determination. If necessary, use background correction.

6.2.2 Quantification by flame AAS

Warm up and stabilize the instrument; then begin measurements.

6.2.3 Calibration and measurement

Use the blank solution (4.6) to zero the instrument. Measure the calibration solutions (4.5.2) and use the extinctions (absorbance) and concentrations to generate a calibration curve. Determine the linear range of the calibration function.

The measurement solution is taken up and measured. Use the calibration curve to convert the determined extinction into concentration units.

For long series of measurements, verify the blank and the calibration function multiple times.

6.3 Graphite furnace atomic absorption spectrometry

6.3.1 General

Set and optimize the instrument according to the manufacturer's specifications. Set the wavelength to 286,3 nm. Use the manufacturer's specifications to set the slit width, volumes of samples and modifiers, and the temperatures and times of the drying, ashing and atomizing steps. Adjust these parameters to the sample matrix to be examined, and optimize the ashing and atomizing times and temperatures in particular.

The instrument parameters depend on the type and amount of modifier used, the type of graphite tube heating and the settings that are recommended by the manufacturer and that result from direct optimization.

NOTE The wavelength of 235,5 nm can be used, but was not tested in the collaborative trial.

6.3.2 Calibration and measurement

6.3.2.1 General

Check if the standard curve and the standard addition curve have comparable slopes. If they deviate significantly, use standard addition.

For samples of unknown composition or sample solutions having a different acid concentration, the standard addition procedure is recommended for calibration.

6.3.2.2 Standard addition procedure

When the standard addition procedure is applied it is important that the measurements are in the linear range. Determine and regularly verify the linear range of the calibration function. At least three measurement points, of which at least two are additions, shall be available for the standard addition procedure. The concentration of the highest addition shall have two to four times the concentration of the sample solution. The concentration of the lowest addition should be approximately half the value of the highest addition.

Plot a straight line of the resulting extinctions against the addition concentrations. Extrapolate the straight line until it intersects the concentration axis.

Use the blank solution to zero the instrument.

Then use defined additions to measure the samples.

NOTE For modern AAS instruments, the autosampler can make the spike addition directly into the graphite tube furnace.

6.3.2.3 Standard calibration procedure

Use the blank solution to zero the instrument.

To set up the calibration function, use the different tin concentrations to measure the extinctions of the calibration solutions (4.5.3). Use pairs of measured values to determine the calibration function.

Measure the sample solution for all canned foods at a dilution of 1:10 or at another suitable dilution. If the extinction lies outside the calibration range or if the sample is too diluted, prepare and measure another dilution.

During dilution, make sure that the acid concentrations in all measurement solutions (sample solutions, blank solutions and calibration solutions) are identical.

For long series of measurements, verify the zero point and the calibration function multiple times.

6.4 Quality Control

For quality control, analyse a reference material with reliably known contents of tin in parallel to each series of measurements. Include all procedural steps starting at the digestion. This also applies to each series of digestions including all procedural steps to prepare and measure blank solutions.

7 Evaluations

7.1 Calculation of the tin content in foodstuffs

Calculate the tin content, w, in milligrams per kilogram of sample or ϕ, in milligrams per litre of sample according to Equation (1):

$$w \text{ or } \phi = \frac{a \times V \times 1\,000 \times F}{E \times 1\,000} \quad (1)$$

where

a is the mass concentration of tin of the measurement solution, in milligrams per litre (mg/l);

V is the volume of the digestion solution diluted to volume, in millilitres (ml);

E is the initial sample mass, in grams (g), or the initial sample volume, in millilitres (ml);

F is the dilution factor (= 1 if the digestion solution was measured without dilution).

7.2 Limit of quantification

The atomic absorption spectrometer should be able to quantify the concentrations in the digestion solution listed in Table 1. The limit of quantification in the digestion solution prepared according to EN 13805 is affected by the flame AAS and, in particular, by the gas settings of the nitrous oxide/acetylene burner. In graphite furnace AAS the graphite tubes, temperature program, type of matrix modifier and amount and type of matrix affect the limit of quantification.

Table 1 — Limits of quantification in the sample measurement solution

Flame AAS	1 mg/l
Graphite furnace AAS (20 µl injection volume)	0,01 mg/l

With regard to the foodstuff, the limit of quantification is dependent on the sampling amount used in the digestion and the final volume of the digestion solution. For trace elements the limit of quantification is conventionally defined as 6σ, where σ is the standard deviation of the field blank signal. Examples are listed in Table 2.

Table 2 — Examples of limits of quantification in the sample

Weighed quantity of sample g	**Final volume** ml	**Mass fraction of tin** mg/kg
0,5	20	40[a]
		0,4[b]
2,0	20	10[a]
		0,1[b]

a Flame AAS.

b Graphite furnace AAS.

8 Precision

8.1 General

Details of an inter-laboratory test 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 in Annex A.

8.2 Repeatability

The absolute difference between two independent single test results obtained with the same test method on identical test material in the same laboratory by the same operator using the same apparatus within a short time interval will exceed the repeatability limit r given in Tables 3 and 4 in not more than 5 % of the cases.

8.3 Reproducibility

The absolute difference between two single test results obtained with the same test method on identical test material in different laboratories by different operators using different equipment will exceed the reproducibility limit R given in Tables 3 and 4 in not more than 5 % of the cases.

Table 3 — Mean value, repeatability and reproducibility for Flame AAS

Sample	$\bar{x}$ mg/kg	r mg/kg	R mg/kg
Carrot puree	230	16,1	45,0
Tomato puree	171	12,4	30,9
Pineapple	259	7,0	32,6
Mixed fruit	72,9	6,7	11,3
Peach powder	82,0	10,6	12,5
Tomato powder	152	11,2	24,5
Beans powder	260	32,6	41,6
Fruit yoghurt powdered	54,7	5,2	14,5
Fish powder	43,2	8,3	15,9

Table 4 — Mean value, repeatability and reproducibility for Graphite furnace AAS

Sample	$\bar{x}$ mg/kg	r mg/kg	R mg/kg
Carrot puree	215	19,2	50,1
Tomato puree	167	28,0	37,6
Pineapple	269	19,0	46,4
Mixed fruit	69,6	7,5	13,7
White wine	2,47	0,11	0,75
Peach powder	85,4	9,7	25,5
Tomato powder	143	8,3	39,1
Beans powdered	246	42,8	55,9
Fruit yoghurt powdered	52,7	9,7	21,0
Fish powder	39,3	3,4	9,2

9 Test report

The test report shall specify at least the following:

a) all information necessary for the complete identification of the sample;

b) the test method used, with reference to this European Standard;

c) the 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) whether the requirement of the repeatability limit has been fulfilled;

g) all operating details not specified in this European Standard or regarded as optional, together with details of any incidents occurred when performing the method which might have influenced the test result(s).

Annex A
(informative)

Results of the inter-laboratory test

The precision of the method was established in 2006 by the Working group "Balanced diets - trace element analysis" established by the Federal Office of Consumer Protection and Food Safety (BVL) for the execution of § 64 of the German Food and Feed Code (LFGB) and by the Working Group "Inorganic Constituents" of the Food Chemistry Society of the German Chemists Society, and has been verified in an inter-laboratory test evaluated in accordance with ISO 5725-1 [3] and ISO 5725-2 [4]. The results are given in Tables A.1 to A.4.

Number of samples "1" means that the results are based on a double determination of one sample. The results under number of samples "2" are based on one result of a blind duplicate sample.

Table A.1 — Statistical results of the inter-laboratory tests for flame AAS for fresh samples

Sample	**Carrot Puree**	**Tomato Puree**	**Pine-apple**	**Mixed Fruit**
Parameter	Fresh samples			
Number of samples	1	1	1	1
Number of laboratories	8	8	8	8
Number of laboratories after elimination of outliers	8	8	8	8
Number of outliers	0	0	0	0
Number of results accepted	16	16	16	16
Mean value $\bar{x}$ (mg/kg)	230	171	259	72,9
Repeatability limit r (mg/kg)	16,1	12,4	7,0	6,7
Repeatability standard deviation s_r (mg/kg)	5,7	4,4	2,5	2,4
Horwitz value r	4,7	4,9	4,6	5,5
Horrat r index	0,53	0,53	0,21	0,58
Reproducibility limit R (mg/kg)	45,0	30,9	32,6	11,3
Reproducibility standard deviation S_R (mg/kg)	15,9	10,9	11,5	4,0
Horwitz value R	7,1	7,4	6,9	8,4
Horrat R index	0,98	0,87	0,64	0,65

Table A.2 — Statistical results of the inter-laboratory tests for flame AAS for lyophilised samples

Sample	Peach Powder	Tomato Powder	Beans Powdered	Fruit yoghurt Powdered	Fish Powder
Parameter	Lyophilised samples				
Number of samples	2	1	2	2	1
Number of laboratories	6	7	8	6	6
Number of laboratories after elimination of outliers	5	7	8	5	6
Number of outliers	1	0	0	1	0
Number of results accepted	10	14	16	10	12
Mean value $\bar{x}$ (mg/kg)	82,0	152	260	54,7	43,2
Repeatability limit r (mg/kg)	10,6	11,2	32,6	5,2	8,3
Repeatability standard deviation s_r (mg/kg)	3,7	4,0	11,5	1,8	2,9
Horwitz value r	5,4	5,0	4,6	5,8	6,0
Horrat r index	0,84	0,52	0,97	0,58	1,13
Reproducibility limit R (mg/kg)	12,5	24,5	41,6	14,5	15,9
Reproducibility standard deviation S_R (mg/kg)	4,4	8,6	16,0	5,1	5,6
Horwitz value R	8,2	7,5	6,9	8,7	9,1
Horrat R index	0,65	0,76	0,82	1,07	1,44

Table A.3 — Statistical results of the inter-laboratory tests for Graphite furnace AAS for fresh samples

Sample	Carrot Puree	Tomato Puree	Pine-apple	Mixed Fruit	White Wine
Parameter	Fresh samples				
Number of samples	1	1	1	1	1
Number of laboratories	8	8	8	8	8
Number of laboratories after elimination of outliers	7	7	8	7	7
Number of outliers	1	1	0	1	1
Number of results accepted	14	14	16	14	14
Mean value $\bar{x}$ (mg/kg)	215	167	269	69,6	2,47
Repeatability limit r (mg/kg)	19,2	28,0	19,0	7,5	0,11
Repeatability standard deviation s_r (mg/kg)	6,8	9,9	6,7	2,6	0,04
Horwitz value r	4,7	4,9	4,6	5,6	9,2
Horrat r index	0,67	1,21	0,55	0,68	0,17
Reproducibility limit R (mg/kg)	50,1	37,6	46,4	13,7	0,75
Reproducibility standard deviation S_R (mg/kg)	17,7	13,3	16,4	4,8	0,27
Horwitz value R	7,1	7,4	6,9	8,4	14,0
Horrat R index	1,15	1,08	0,89	0,82	0,77

Table A.4 — Statistical results of the inter-laboratory tests for Graphite furnace AAS for lyophilised samples

Sample	**Peach Powder**	**Tomato Powder**	**Beans Powdered**	**Fruit yoghurt Powdered**	**Fish Powder**
Parameter	Lyophilised samples				
Number of samples	2	1	2	2	1
Number of laboratories	8	8	8	8	8
Number of laboratories after elimination of outliers	7	6	7	6	5
Number of outliers	1	2	1	2	3
Number of results accepted	14	12	14	12	10
Mean value $\bar{x}$ (mg/kg)	85,4	143	246	52,7	39,3
Repeatability limit r (mg/kg)	9,7	8,3	42,8	9,7	3,4
Repeatability standard deviation s_r (mg/kg)	3,4	2,9	15,1	3,4	1,2
Horwitz value r	5,4	5,0	4,6	5,8	6,1
Horrat r index	0,74	0,41	1,33	1,12	0,51
Reproducibility limit R (mg/kg)	25,5	39,1	55,9	21,0	9,2
Reproducibility standard deviation S_R (mg/kg)	9,0	13,8	19,8	7,4	3,2
Horwitz value R	8,2	7,6	7,0	8,8	9,2
Horrat R index	1,29	1,27	1,15	1,59	0,89

Table A.5 — Trueness results for Flame AAS

Sample	**Mean value** mg/kg	**Reproducibility standard deviation** S_R mg/kg	**Assigned value** mg/kg	**Confidence interval** (95 %) mg/kg	**Z-Score**
Carrot Puree	230	16	222	32	0,5
Tomato Puree	171	11	162	24	0,7
Mixed Fruit	72,9	4,0	69,5	19	0,4
Beans Powdered	260	16	249	24	0,8

Table A.6 — Trueness results for Graphite furnace AAS

Sample	**Mean value** mg/kg	**Reproducibility standard deviation** S_R mg/kg	**Assigned value** mg/kg	**Confidence interval** (95 %) mg/kg	**Z-Score**
Carrot Puree	215	18	222	32	-0,4
Tomato Puree	167	13	162	24	0,4
Mixed Fruit	69,6	4,8	69,5	19	0
White Wine	2,47	0,27	2,38	0,25	0,6
Beans Powdered	246	20	249	24	- 0,2

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[3] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[4] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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