**BS EN 14546:2005**

# Foodstuffs — Determination of trace elements — Determination of total arsenic by hydride generation atomic absorption spectrometry (HGAAS) after dry ashing

The European Standard EN 14546:2005 has the status of a British Standard

ICS 67.050

British Standards

# National foreword

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English version

# Foodstuffs - Determination of trace elements - Determination of total arsenic by hydride generation atomic absorption spectrometry (HGAAS) after dry ashing

Produits alimentaires - Détermination des éléments traces - Détermination de l'arsenic total par spectrométrie d'absorption atomique par génération d'hydrures (SAAGH) après calcination par voie sèche

Lebensmittel - Bestimmung von Elementspuren - Bestimmung von Gesamtarsen mit Atomabsorptionsspektrometrie-Hydridtechnik (HGAAS) nach Trockenveraschung

This European Standard was approved by CEN on 15 March 2005.

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# Contents

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# Foreword

This document (EN 14546:2005) 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of an identical text or by endorsement, at the latest by October 2005, and conflicting national standards shall be withdrawn at the latest by October 2005.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

# 1 Scope

This European Standard specifies a method for the determination of total arsenic in foodstuffs by hydride generation atomic absorption spectrometry (HGAAS) after dry ashing.

Specific foodstuffs for which European Standards exist are excluded from the scope of this horizontal European Standard. It is the task of the analyst to review if vertical standards exist.

# 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 13804, *Foodstuffs – Determination of trace elements – Performance criteria, general considerations and sample preparation.*

# 3 Principle

The samples treated with nitric acid and ashing aid are evaporated to dryness and then mineralized at 425 °C with a gradual increase in temperature. The ash is dissolved in hydrochloric acid and arsenic is quantified by HGAAS-procedure at the arsenic line at 193,7 nm [1].

**WARNING — The use of this standard may 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 health and safety practices and determine the applicability of regulatory limitations prior to use.**

# 4 Reagents

## 4.1 General

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

## 4.2 Hydrochloric acid

**4.2.1 Hydrochloric acid,** not less than 30 % of approximately $\rho$ (HCl) = 1,15 g/ml.

**4.2.2 Diluted hydrochloric acid,** c ( ≈ 6 mol/l).

Dilute 500 ml hydrochloric acid (4.2.1) with water in a proportion of 1+1 parts by volume.

### 4.3 Nitric acid, not less than 65 % (mass fraction) of approximately $p$ ($HNO_3$) =1,4 g/ml.

#### 4.3.1 Diluted nitric acid 1, w = 32 %1)

To be prepared by mixing mix nitric acid (4.3) and water in a proportion of 1 + 1 parts by volume.

#### 4.3.2 Diluted nitric acid 2, w = 6,5 %1)

Mix nitric acid (4.3) and water in a proportion of 1 + 9 parts by volume.

#### 4.3.3 Diluted nitric acid 3, w = 3,3 %1)

Mix nitric acid (4.3) and water in a proportion of 1 + 19 parts by volume.

### 4.4 Magnesium oxide, w = not less than 90 %1)

### 4.5 Magnesium nitrate hexahydrate, w = not less than 98 %1)

### 4.6 Ashing aid

Suspend 20 g of magnesium nitrate hexahydrate (4.5) and 2 g of magnesium oxide (4.4) in 100 ml of water.

### 4.7 Sodium hydroxide, w = not less than 95 %1)

### 4.8 Potassium iodide, w = not less than 99,5 %1)

### 4.9 Ascorbic acid, w =not less than 99,7 %1)

### 4.10 Pre-reducing solution

Dissolve 5 g of potassium iodide (4.8) and 5 g of ascorbic acid (4.9) in 100 ml of water.

### 4.11 Arsenic stock solution, in nitric acid, with a mass concentration ρ(As (V))=1 000 mg/l

The use of a stock solution accompanied by a certificate is advisable.

### 4.12 Arsenic calibration solutions

#### 4.12.1 Intermediate standard solution

Prepare a standard of 25 µg/l in HCl (4.2.2) from the stock solution (4.11)

#### 4.12.2 Calibration standards

Take an aliquot of the intermediate standard (e.g. 3 ml for a final concentration of 3 µg/l) and make up to 5 ml with HCl (4.2.2). Add 5 ml of pre-reducing solution (4.10). Wait 30 min and make up to 25 ml with HCl (4.2.2). Following the same procedure prepare at least three calibration standards at different concentrations plus the zero point of the curve. The concentrations selected shall not exceed the linear range of the calibration curve of arsenic.

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1) *w* is the mass fraction.

### 4.13 Sodium borohydride, w = not less than 96%[1)]

### 4.14 Sodium hydroxide, w = not less than 98%[1)]

### 4.15 Sodium borohydride solution

The concentration of this reducing solution may vary according to the system of hydride generation employed and the recommendations given by the manufacturer in the manual for the equipment. Filter if necessary. This solution shall be prepared daily.

## 5 Apparatus and equipment

### 5.1 General

To minimize the contamination, all apparatus which come into direct contact with the sample and the solutions shall be carefully pre-treated according to EN 13804.

### 5.2 Programmable muffle furnace

Equipped with a microprocessor or muffle furnace with thermostat.

### 5.3 Atomic absorption spectrometer

Equipped with an electrically heated quartz cell and an accessory for hydride generation with or without flow injection.

### 5.4 Lamp for arsenic

Hollow-cathode or electrodeless discharge lamp.

### 5.5 Hot plate or sand bath

With stepwise heating control.

## 6 Procedure

### 6.1 Pre-treatment

Homogenise the sample in accordance with EN 13804.

### 6.2 Test portion

Weigh an amount of 250 mg to 1000 mg dry sample or an equivalent quantity of fresh sample into a tall heat resistant glass beaker with a minimum capacity of 200 ml.

### 6.3 Dry ashing

#### 6.3.1 Ashing procedure

Add 2,5 ml of ashing aid (4.6) per 250 mg of dry sample. In order to avoid precipitation add the ashing aid while shaking the suspension. Then add 5 ml of nitric acid (4.3.1) and evaporate in a sand bath (5.5) to a dry residue. Increase the temperature gradually and slowly to avoid boiling, the production of foam, and splashing or spattering.

Cover the glass beaker with a watch glass and place in the muffle furnace at an initial temperature not higher than 150 °C. Increase the temperature to (425 ± 25) °C at a maximum rate of 50 °C/h and maintain for 12 h. The rate of increase in temperature shall be maintained at the level recommended to avoid a loss of material. Take the crucible out of the furnace and let it cool.

Repeat the ashing procedure until the sample is completely incinerated. For this purpose add 5 ml of nitric acid (4.3.2), evaporate in the sand bath, and place again the ashes in the muffle furnace, i.e. the ashes should be white/grey or slightly coloured (the number of repetitions necessary varies depending on the type of sample).

### 6.3.2 Dissolution of the sample ash

Add 1 ml of water to wet the ash and then add 5 ml of hydrochloric acid (4.2.2) to the crucible, ensuring that all the ash comes into contact with the acid. Then add 5 ml of pre-reducing agent (4.10). Wait 30 min and make up to 25 ml with diluted hydrochloric acid (4.2.2).

If necessary, filter the samples before diluting to volume. The filter shall be decontaminated by an initial washing with nitric acid (4.3.3) and two subsequent washings with deionized water. After filtering the samples and in order to avoid losses, wash the filter with hydrochloric acid (4.2.2) and add the washing liquids to the solution before diluting to volume, as above.

Treat three reagent blanks in the same way as the samples.

The sample solutions are stable for 24 h at 4 °C.

## 6.4 Hydride generation atomic absorption spectrometry (HGAAS)

### 6.4.1 Spectrometer conditions

Optimize the instrument in accordance with the recommendations described in the manual provided with the instrument. The readings shall be made at 193,7 nm.

### 6.4.2 Hydride generation technique

#### 6.4.2.1 General

Certain elements (e.g. Co, Cu, Cr, Fe, Mg, Ni, Sb, Sn and Se) may cause interference in hydride generation. This interference is limited, or eliminated, when the sample is diluted in diluted hydrochloric acid (4.2.2). If the sample requires further dilution, do so with diluted hydrochloric acid (4.2.2). Confirm that there are no matrix effects or inter-element interference by verifying that the differences between the slopes of the calibration curves and the standard addition curves are not significant. If the matrix effect persists, use the method of standard addition.

#### 6.4.2.2 Standard addition method

Determine the linear range of the standard calibration function. It is important that the measurements are made in the linear range when the method of standard addition is used. A standard addition curve should consist of at least three points of which at least two are standard additions. The concentration of the highest standard should be 3 to 5 times the concentration in the sample solution. The concentration of the lower standard should be half of the highest standard.

Plot a graph of the absorbances obtained in this way against the added concentrations and extrapolate the resulting straight line until it intercepts the concentration axis.

## 7 Calculation

Calculate the mass fraction, $w$, of total arsenic, in milligram per kilogram of the sample, using equation (1):

$$w = \frac{a \times V \times F}{m \times 1000} \qquad (1)$$

where

$a$ is the concentration of the element to be determined in the test solution used, in microgram per litre;

$V$ is the volume of the sample solution, in millilitre;

$F$ is the dilution factor, taking into account the pre-reduction and any further dilutions in the case of high element concentrations;

$m$ is the initial sample mass, in gram.

If the concentration in the blank solutions is below the limit of quantification of the method, it shall not be considered in the calculations. If necessary, subtract the concentration in the blank solution from the concentration of the sample solution.

## 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.

### 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 Table 1 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 Table 1 in not more than 5 % of the cases.

**Table 1 — Mean values repeatability limits and reproducibility limits**

| Sample | $\bar{x}$ (mg/kg) | *r* (mg/kg) | *R* (mg/kg) |
|---|---|---|---|
| Spinach leaves (SRM 1570a, NIST) | 0,088 | 0,058 | 0,109 |
| Lobster hepatopancreas (TORT-2, NRCC) | 18,6 | 2,5 | 10,5 |
| Dogfish liver (DOLT-2, NRCC) | 14,6 | 3,8 | 7,3 |
| Rice flour (GBW8502, China) | 0,056 | 0,018 | 0,032 |
| Wheat flour (GBW8503, China) | 0,196 | 0,039 | 0,080 |

## 9 Test report

The test report shall specify at least the following:

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

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

c) 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 working group AEN/CTN34/GT1 “Trace elements (heavy metals)” of the Spanish Standardisation Institute (AENOR) established the precision of the method in 2000. The method has been verified in an inter-laboratory test [1] and evaluated in accordance with ISO 5725-2:1994. The statistical results are given in Table A.1. The certified values are given in Table A.2.

**Table A.1 — Statistical results of the inter-laboratory tests**

| Parameter | Spinache leaves | Lobster hepatopancreas | Dogfish liver | Rice flour | Wheat flour |
|---|---|---|---|---|---|
| Number of laboratories | 10 | 10 | 10 | 10 | 10 |
| Number of laboratories after elimination of outliers | 8 | 8 | 10 | 9 | 9 |
| Number of outliers | 2 | 2 | 0 | 1 | 1 |
| Mean value $\bar{x}$ (mg/kg) | 0,088 | 18,6 | 14,6 | 0,056 | 0,196 |
| Repeatability standard deviation $s_r$ (mg/kg) | 0,021 | 0,9 | 1,3 | 0,007 | 0,014 |
| $RSD_r$ (%) | 23,6 | 4,8 | 9,2 | 11,7 | 7,1 |
| Repeatability limit $r$ (mg/kg) | 0,058 | 2,5 | 3,8 | 0,018 | 0,039 |
| Horwitz value r | 15 | 7 | 7 | 16 | 14 |
| Horrat $r$ index | 1,53 | 0,7 | 1,3 | 0,71 | 0,52 |
| Reproducibility standard deviation $S_R$ (mg/kg) | 0,039 | 3,8 | 2,6 | 0,011 | 0,028 |
| $RSD_R$ (%) | 44,4 | 20,3 | 17,8 | 20,1 | 14,5 |
| Reproducibility limit $R$ (mg/kg) | 0,109 | 10,5 | 7,3 | 0,032 | 0,080 |
| Horwitz value $R$ | 23 | 10 | 11 | 25 | 20 |
| Horrat $R$ index | 1,93 | 2,0 | 1,7 | 0,81 | 0,71 |

**Table A.2 — Certified reference materials, Z-score evaluation of found values against certified values**

| **Standard Reference Material** | **Found mean value**<br>mg/kg | **n** | $S_R$ | **Certified mean value**<br>mg/kg | **95% confidence interval** | **Z-score**[a] |
|---|---|---|---|---|---|---|
| Spinach leaves (SRM 1570a, NIST) | 0,088 | 8 | 0,039 | 0,068 | 0,012 | 1,3 |
| Lobster hepatopancreas (TORT-2, NRCC) | 18,6 | 8 | 3,8 | 21,6 | 1,8 | -1,9 |
| Dogfish liver (DOLT-2, NRCC) | 14,6 | 10 | 2,6 | 16,6 | 1,1 | -2,0 |
| Rice flour (GBW8502, China) | 0,056 | 9 | 0,011 | 0,051 | 0,005 | 1,1 |
| Wheat flour (GBW8503, China) | 0,196 | 9 | 0,028 | 0,22 | 0,04 | -1,1 |

[a] Z-score according to NMKL procedure No 9 [2]

# Bibliography

ISO 5725-2:1994, *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*

[1] Arbitral Agroalimentary Laboratory (General Subdirectorate of the Quality Foodstuffs Control, Ministry of Agriculture Fisheries and Food) in collaboration with the Institute of Agrochemistry and Food Technology (Spanish Scientific Research Council). The results obtained are collected in the document AEN/CTN 34/SC4/GT1/Nº 28.

[2] NMKL Procedure No 9. Evaluation of results derived from the analysis of certified reference materials. (2001). Nordic Committee on Food Analysis. C/o National Veterinary Institute, Box 8156 Dep. 0033 Oslo, Norway.

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