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Foodstuffs — Determination of trace elements — Determination of inorganic arsenic in seaweed by hydride generation atomic absorption spectrometry (HGAAS) after acid extraction

ICS 67.050

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Foreword

This document (EN 15517:2008) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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1 Scope

This document specifies a procedure for the determination of hydrochloric acid (gastric acid concentration) extractable inorganic arsenic in seaweed. Collaborative studies have been carried out (Annex A). The method is suitable for the determination of inorganic arsenic not less than 1 mg/kg and below 100 mg/kg on a dry weight basis. The amount of inorganic arsenic is considered to be that part determined by the procedure described in this document.

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

Arsenic compounds are extracted from the sample by diluted hydrochloric acid (in accordance with EN 71-3 [5]) and the arsenic in the extract is determined by hydride generation AAS. In acidic media inorganic compounds of arsenic(III) and arsenic(V) as well as the monomethylarsonic acid (MMA) and dimethylarsinic (cacodylic) acid (DMA) form a volatile hydride with sodium borohydride. There is no reaction of the stable organic arsenic compounds like arsenosugar, arseno-betaine and arseno-choline under these conditions. The gaseous hydride is transferred into a heated measuring cell (cuvette) by means of a carrier gas stream and decomposed. The absorption at 193,7 nm (arsenic line) serves as a measure of arsenic concentration. The hydride signal sensitivity of DMA reaches generally low rates as compared to As(III). The contribution of MMA in the hydride signal can be neglected, since MMA occurs in seaweed only in small amounts. The hydride generation AAS in combination with this hydrochloric acid extraction may be used as nearly selective method of determination for inorganic arsenic.

Generation of arsine from As(III) is much faster and gives greater sensitivity than generation from As(V) and is also less subject to interference. Arsenic(V) shall be reduced to arsenic(III) (pre-reduction) in order to avoid incorrect measurements.

4 Reagents

4.1 General

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

4.2 Hydrochloric acid, mass fraction w = 30 %, mass concentration ρ(HCl) = 1,15 g/ml.

4.3 Hydrochloric acid solution, substance concentration c = (0,07 ± 0,005) mol/l.

4.4 Hydrochloric acid, approximately c = 2 mol/l.

4.5 Sodium borohydride, $w \geq 96$ %

4.6 Sodium hydroxide, $w \geq 98$ %

4.7 Sodium borohydride solution, e.g. substance concentration c = 2 g/l (example for using the flow injection procedure described under 6.2.1 (b)).

Dissolve 2 g of sodium hydroxide pellets in water, add 2 g of sodium borohydride and dilute to 1 000 ml with water.

A fresh solution shall be prepared daily and filtered before use.

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.

4.8 Diluted hydrochloric acid, e.g. mass fraction $w \approx 3$ % (carrier solution, only for use in the flow injection procedure).

Dilute approximately 90 ml of hydrochloric acid (4.2) to 1 000 ml with water.

The concentration by mass of the carrier solution may vary with the system and the instructions of the relevant manufacturer shall therefore be observed.

4.9 L-Ascorbic acid, $w(C_6H_8O_6) \geq 99,7$ %

4.10 Potassium iodide, w(KI) ≥ 99,5 %

4.11 Potassium iodide/ascorbic acid solution

Dissolve 3 g of potassium iodide and 5 g of ascorbic acid in water and dilute to 100 ml.

Prepare a fresh solution daily.

The concentrations of the potassium iodide and ascorbic acid may vary slightly with the system and the instructions of the relevant manufacturer shall therefore be observed.

4.12 Diarsenic trioxide (As2O3), $w(As_2O_3) \geq 99,5$ %

4.13 Arsenic stock solution, with an arsenic mass concentration of 1000 mg/l.

If commercial stock solutions are not available, proceed as follows: dissolve 1,320 g of diarsenic trioxide

(4.12) in 25 ml of potassium hydroxide solution (ρ = 20 g/100 ml), neutralize with 20 % (mass fraction) sulfuric acid with phenolphthalein as indicator and dilute to 1 000 ml with 1 % (mass fraction) sulfuric acid.

4.14 Arsenic standard solutions

Dilute the arsenic stock solution (4.13) in several steps. The arsenic standard solutions shall contain an adequate amount of hydrochloric acid, e.g. 2 ml of hydrochloric acid (4.2) per 100 ml.

Example of a dilution series:

$$1000\ \text{mg/l} \xrightarrow[5/100]{} 50\ \text{mg/l} \xrightarrow[5/50]{} 5\ \text{mg/l} \xrightarrow[1/50]{} 0{,}1\ \text{mg/l}$$

A standard solution of 5 mg/l arsenic in 0,6 % (mass fraction) hydrochloric acid is stable for at least one week.

4.15 Arsenic calibration solutions

Prepare five calibration solutions in the required calibration range from the standard solution of 0,1 mg/l (4.14), ensuring that the concentrations of the calibration solutions are not outside the linear range of the calibration function and are also in the expected sample content range. The concentration of acid in the calibration solutions shall be equal to that in the sample solution.

Example for the 1 µg/l to 10 µg/l range:

$$0{,}1\ \text{mg/l} \xrightarrow[1/100]{} 1\ \mu\text{g/l}$$

$$\xrightarrow[3/100]{} 3\ \mu\text{g/l}$$

$$\xrightarrow[5/100]{} 5\ \mu\text{g/l}$$

$$\xrightarrow[8/100]{} 8\ \mu\text{g/l}$$

$$\xrightarrow[10/100]{} 10\ \mu\text{g/l}$$

The calibration solutions may also be prepared from the appropriately diluted standard solution in the measurement vessel itself by adding the reagents for the pre-reduction (see 6.1.3).

Prepare fresh calibration solutions daily.

The following procedure is recommended for the preparation of standard and calibration solutions: pour some water into the volumetric flask and add the requisite amount of acid. After cooling to room temperature, add the stock or standard solution using a pipette and dilute to the mark with water.

4.16 Zero member compensation solution, containing water and acid in a concentration equal to that in the sample solution.

5 Apparatus and equipment

5.1 General

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

5.2 Atomic absorption spectrometer, with measurement recording system and accessories for the hydride generation method.

5.3 Element-specific lamp (hollow-cathode or electrodeless discharge lamp) for arsenic.

5.4 Centrifuge

5.5 Syringe filter (unit), pore size 0,45 µm, diameter 25 mm, resistant to hydrochloric acid (4.4). Membranes of polyester or nylon have been proven suitable.

5.6 Indicator paper

5.7 Device for thermostating, at approximately 37 °C.

5.8 Stirrer or shaking machine

6 Procedure

6.1 Sample preparation

6.1.1 General

It is possible that extracted arsenic compounds are decomposed to inorganic arsenic, even when stored in a refrigerator. Therefore, determination by hydride generation AAS should be conducted as soon as possible, latest within one week.

6.1.2 Hydrochloric acid extraction

In imitation of EN 71-3 the well homogenized sample is weighed (minimum weighed portion 0,2 g) into a vessel, which is suitable for the extraction and allows sufficient agitation motion. The ratio of weighed portion to extracting agent (extractant) shall be 1:50 (1 part + 49 parts). Add the appropriate amount of hydrochloric acid solution (4.3) of approximately 37 °C to the sample and mix for 1 min. Transfer one drop of this mixture onto indicator paper. If the pH is more than 1,5, add dropwise hydrochloric acid (4.4) while stirring until the pH value lies between 1,0 and 1,5. Continuously agitate (by stirring or shaking) the suspension at a temperature of approximately 37 °C for 1 h and then allow it to stand for 1 h at approximately 37 °C. Immediately afterwards solids shall be separated from the solution. First centrifuge for 10 min and then filter through a syringe filter (5.5). The extract shall be free of particles. The concentration of arsenic in the solution should be measured by hydride generation AAS as soon as possible. The extraction solution is stored in a suitable vessel in a refrigerator until measurement.

6.1.3 Pre-reduction

Depending on the hydride system used, it may be necessary to use larger or smaller volumes than described below. The ratios specified shall, however, be maintained.

Introduce and thoroughly mix 2 ml of calibration solution (4.15) and 2 ml of hydrochloric acid (4.2) into the measurement vessel of the hydride system. Then add 1 ml of potassium iodide/ascorbic acid solution (4.11) and again mix thoroughly. After leaving for 45 min at room temperature in an open vessel, dilute to 10 ml with water and mix thoroughly to obtain a solution ready for measuring. If the calibration solution is prepared in the measurement vessel itself, use the appropriate quantity of standard solution and dilute to 2 ml with the zero member compensation solution (4.16), then proceed as described above.

Treat the zero member compensation and the sample solutions in the same way. Up to 2 ml hydrochloric acid extract according to 6.1.2 are used for the pre-reduction. If necessary, the dilutions are made with zero member compensation solution (4.16) prior to the pre-reduction. Compensate using less than 2 ml of sample solution by adding the appropriate amount of zero member compensation solution (4.16).

The acid and reducing-agent concentrations shall be the same in all the test solutions.

Only seal the measurement vessels after diluting with water for the final mixing. Do not analyse yellow-coloured solutions as they give incorrect results (too low or too high). To avoid problems in the pre-reduction, do not pipette more than 20 solutions in one series.

IMPORTANT — The proportion of the inorganic arsenic can be up to 90 % of the total arsenic content in the sample. It has been proven convenient to determine first the total arsenic content of the sample in order to use an appropriate dilution of the hydrochloric acid extract for the pre-reduction. Since the measurement solution may foam in the hydride system, take care when using non-diluted hydrochloric acid extracts for pre-reduction!

6.2 Atomic absorption spectrometry (hydride generation AAS)

6.2.1 Working conditions for AAS and hydride generation

The following two methods are recommended in the hydride procedure.

a) Continuous flow system, in which samples and reagents are reacted until a time-independent signal is produced.

b) Flow-injection method, in which the sample is added to the carrier solution acidified with hydrochloric acid by means of a sample loop, whose volume is optional, and reacted in the mixing unit with the reducing agent, resulting in a time-dependent signal.

The different sample volumes used in these methods result in different detection limits.

Sodium borohydride (4.5) is used as reducing agent in very different concentrations and amounts to suit the method employed.

Adjust the apparatus as specified in the manufacturer's operating manual and determine an optimum test schedule.

6.2.2 AAS measurement

Use only the pre-treated solutions (6.1.3) for all measurements.

Zero the instrument with the zero member compensation solution (4.16).

To plot the calibration function, measure the absorbances of the calibration solutions with different concentrations of the element and determine the calibration function from the pairs of measurements. Ensure that the calibration function is in the linear range. Measure the sample solution directly or after suitable dilution (as described under 6.1.3), if the absorbance is outside the calibration range.

In the case of fairly long measurement series, repeatedly check the zero and calibration function.

NOTE 1 In order to ensure quality, the extraction yield should be checked by determination of total arsenic content in the sample extract. For this a measurement method should be applied, which detects the total arsenic content regardless of still present organic-bound arsenic. This may be done e.g. either by graphite-furnace AAS [1] or by ICP-OES or ICP-MS.

NOTE 2 Hydride generation AAS [2] is only applicable for the determination of total arsenic in marine samples after complete destruction of organic arsenic compounds. This can be achieved e.g. by pressure digestion with nitric acid at temperatures of at least 320 °C [3].

NOTE 3 The extraction yields for seaweed are usually in the range from 70 % to 90 %. In individual cases, depending on the kind of seaweed, yields can be considerably lower.

7 Calculation

Calculate the content w of inorganic arsenic according to clause 1, in milligram per kilogram of sample, using equation (1):

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

where

a is the amount of arsenic in the measurement solution used, measured by HGAAS, in microgram per litre;

V is the volume of the hydrochloric acid solution used for extraction, in millilitres;

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

m is the weighed sample portion for extraction, in grams.

8 Precision

8.1 General

Details of an inter-laboratory test on the precision of the method are summarized 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 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 repeatability limit r in Table 1.

8.3 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 reproducibility limit R in Table 1.

Table 1 — Repeatability and reproducibility for inorganic arsenic

Sample	Mean value $\bar{x}$ mg/kg	Repeatability limit *r* mg/kg	Reproducibility limit *R* mg/kg
Sechee	0,08	0,03	0,09
BCR 279 Sea Lettuce	0,85	0,14	0,55
Black Moss	2,83	0,64	1,04
Hijiki B	31,8	3,55	5,76
Hijiki A	72,9	7,41	12,2
Hijiki C	80,2	7,36	9,99

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 and the element to be determined, with reference to this document;

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

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

Annex A
(informative)

Results of the inter-laboratory tests

This method has been prepared by the working group "Bilanzierte Diäten — Spurenelementanalyse" (Balanced diets — Analysis of trace elements) of the commission of the former federal health department (Bundesgesundheitsamt) for the implementation/execution of § 64 LFGB and by the working group "Anorganische Bestandteile" (Inorganic Components) of the special division "Lebensmittelchemische Gesellschaft" (Food-chemical Society) within the Society of German Chemists. It has been tested in an inter-laboratory test with 13 participants altogether in accordance with ISO 5725-1 [7].

Table A.1 — Statistical values for total arsenic in sample

Parameter	total arsenic in sample					
	Sechee	BCR 279 Sea Lettuce	Black Moss	Hijiki B	Hijiki A	Hijiki C
Number of laboratories	13	13	13	13	13	13
Number of laboratories after elimination of outliers	12	11	12	11	12	11
Number of outliers	1	2	1	2	1	2
Mean value $\bar{x}$, mg/kg	34,5	2,69	26,9	68,0	107	112
Repeatability limit *r,* mg/kg	4,78	0,52	4,08	7,76	15,49	14,27
Repeatability standard deviation s_r, mg/kg	1,71	0,19	1,46	2,77	5,53	5,10
Horwitz value *r*	6,20	9,10	6,43	5,60	5,23	5,19
Horrat *r* index	0,8	0,8	0,8	0,7	1,0	0,9
Reproducibility limit *R,* mg/kg	11,1	0,98	14,1	14,4	40,1	19,9
Reproducibility standard deviation S_R, mg/kg	3,97	0,35	5,04	5,14	14,33	7,11
Horwitz value *R*	9,39	13,8	9,75	8,48	7,92	7,86
Horrat *R* index	1,2	0,9	1,9	0,9	1,7	0,8

Table A.2 — Statistical values for total arsenic in HCl-extract

Parameter	total arsenic in HCl-extract					
	Sechee	BCR 279 Sea Lettuce	Black Moss	Hijiki B	Hijiki A	Hijiki C
Number of laboratories	7	7	7	7	7	7
Number of laboratories after elimination of outliers	7	6	7	7	7	7
Number of outliers	0	1	0	0	0	0
Mean value $\bar{x}$, mg/kg	34,6	1,99	3,61	55,4	95,0	102
Repeatability limit *r,* mg/kg	2,94	0,56	1,07	4,17	7,09	6,13
Repeatability standard deviation s_r, mg/kg	1,05	0,20	0,38	1,49	2,53	2,19
Horwitz value *r*	6,19	9,52	8,70	5,77	5,32	5,26
Horrat *r* index	0,5	1,1	1,2	0,5	0,5	0,4
Reproducibility limit *R,* mg/kg	10,8	0,94	2,27	8,69	19,3	19,4
Reproducibility standard deviation S_R, mg/kg	3,88	0,34	0,81	3,10	6,88	6,92
Horwitz value *R*	9,38	14,4	13,2	8,74	8,06	7,97
Horrat *R* index	1,2	1,2	1,7	0,6	0,9	0,8
Extraction yields calculated from the mean values from table A.1 and A.2, %	100	74	13	81	89	91

Table A.3 — Statistical values for inorganic arsenic in HCl-extract

Parameter	inorganic arsenic in HCl-extract					
	Sechee	BCR 279 Sea Lettuce	Black Moss	Hijiki B	Hijiki A	Hijiki C
Number of laboratories	7	9	9	9	9	9
Number of laboratories after elimination of outliers	6	9	8	8	7	8
Number of outliers	1	0	1	1	2	1
Mean value $\bar{x}$, mg/kg	0,08	0,85	2,83	31,8	72,9	80,2
Repeatability limit *r,* mg/kg	0,03	0,14	0,64	3,55	7,41	7,36
Repeatability standard deviation s_r, mg/kg	0,01	0,05	0,23	1,27	2,65	2,63
Horwitz value *r*	15,4	10,8	9,03	6,27	5,54	5,46
Horrat *r* index	0,8	0,5	0,9	0,6	0,7	0,6
Reproducibility limit *R,* mg/kg	0,09	0,55	1,04	5,76	12,2	9,99
Reproducibility standard deviation S_R, mg/kg	0,03	0,20	0,37	2,06	4,34	3,57
Horwitz value *R*	23,4	16,4	13,7	9,51	8,39	8,27
Horrat *R* index	1,6	1,4	1,0	0,7	0,7	0,5

The interlaboratory test has shown that the method for determination of inorganic arsenic described complies with the sum of (the results for) arsenic(III)/arsenic(V) from speciation analyses (chromatographic separation and ICP-MS detection) [4].

No certified reference materials for inorganic arsenic were available. During the inter-laboratory trial the accuracy of the method was controlled by IC-ICP-MS speciation analysis of the analysed samples' water extract. Testing accuracy by IC-ICP-MS was possible only by the use of aqueous algae extracts. Hydrochloric acid extracts could not be analysed by chromatographic speciation analysis. Therefore Table A.4 compares the results of aqueous extracts.

Table A.4 — Accuracy results obtained in the water extract

Sample	Inorganic As by H-AAS mg/kg	± SD	Sum of As3+/5+ by IC-ICP-MS mg/kg	± SD	Z-Score
Hijiki A	66,2	8,9	57	15,7	1,1
Hijiki B	30	3,0	28	2,5	1,2
Hijiki C	73,3	7,9	73	7,1	0,1
Black Moss	0,39	0,12	0,72	0,65	-1,0
BCR 279 Sea Lettuce	0,43	0,1	0,47	0,11	-0,7

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[5] EN 71-3, Safety of toys — Part 3: Migration of certain elements

[6] Schuffenhauer, C., Bestimmungsmethode für anorganisches Arsen in Algen, Lebensmittelchemie 56, pp. 100 ff, (2002)

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