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Foodstuffs — Determination of elements and their chemical species — Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS

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Foodstuffs - Determination of elements and their chemical species - Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS

Produits alimentaires - Détermination des éléments et de leurs espèces chimiques - Détermination de la teneur en arsenic inorganique dans les produits alimentaires d'origines marine et végétale, par CLHP avec échange d'anions et spectrométrie de masse à plasma induit par haute fréquence (ICP-SM)

Lebensmittel - Bestimmung von Elementen und ihren Verbindungen - Bestimmung von anorganischem Arsen in Lebensmitteln marinen Ursprungs und pflanzlichen Lebensmitteln mit Anionenaustausch-HPLC-ICP-MS

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European foreword

This document (EN 16802:2016) 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of an identical text or by endorsement, at the latest by September 2016, and conflicting national standards shall be withdrawn at the latest by September 2016.

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1 Scope

This European Standard describes a procedure for the determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS following waterbath extraction.

This method has been validated in an interlaboratory test on white rice, wholemeal rice, leek, blue mussels, fish muscle and seaweed with an inorganic arsenic mass fraction in the range 0,073 mg/kg to 10,3 mg/kg [1].

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 13804, *Foodstuffs — Determination of elements and their chemical species — General considerations and specific requirements*

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696)*

3 Principle

This standard describes a method for the determination of inorganic arsenic. Inorganic arsenic consists of arsenite, As(III) and arsenate, As(V). A representative test portion of the sample is treated with a diluted nitric acid and hydrogen peroxide solution in a heated waterbath. Hereby the arsenic species are extracted into solution and As(III) is oxidized to As(V). The inorganic arsenic is selectively separated from other arsenic compounds using anion exchange HPLC (High Performance Liquid Chromatography) coupled online to the element-specific detector ICP-MS (Inductively Coupled Plasma Mass Spectrometry) for the determination of the mass fraction of inorganic arsenic. External calibration with solvent matrix-matched standards is used for quantification of the amount of inorganic arsenic.

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 its use.

4 Reagents

4.1 General

The concentration of the arsenic species in the reagents and water used shall be low enough to not affect the results of the determination. When using a method of high sensitivity like ICP-MS, the control of the blank levels of water, acid and other reagents is very important. Generally ultra-pure water complying with ISO 3696 grade 1 (i.e. electrical conductivity below 0,1 µS/cm at 25 °C) and acid of high purity, e.g. cleaned by sub-boiling distillation, are recommended. Reagents should be of minimum p.a. quality where possible. Special facilities can be used in order to avoid contamination during the steps of preparation and measurement (e.g. uses of laminar flow benches or comparable clean room facilities).

4.2 Nitric acid concentrated, mass fraction $w(HNO_3)$, ≥ 65 %, mass concentration of approximately $\rho(HNO_3)$ = 1,4 g/ml.

Use only nitric acid available with high purity or perform a clean-up by a sub-boiling distillation in order to avoid potential contamination.

4.3 Hydrogen peroxide, $w(H_2O_2) \geq 30$ %.

High purity is essential to avoid potential contamination. Commercially available hydrogen peroxide for analysis should be tested for contamination of arsenic.

4.4 Extraction solution 1, $c(HNO_3) = 0{,}1$ mol/l in 3 %(V/V) H_2O_2.

Pour 800 ml of H_2O and then 6,5 ml of HNO_3 (4.2) and thereafter 100 ml of H_2O_2 (4.3) into a 1 000 ml volumetric flask. Fill it up to the mark with H_2O. This solution should be prepared on the same day of use.

It is recommended that the total volume needed for the analysis is estimated and only this amount is produced.

4.5 Extraction solution 2, $c(HNO_3) = 0{,}2$ mol/l in 6 % (V/V) H_2O_2.

Pour 70 ml of H_2O, 1,3 ml of nitric acid (4.2) and 20 ml of hydrogen peroxoide (4.3) into a 100 ml volumetric flask. Fill up to the mark at 100 ml with H_2O. This solution should be prepared on the same day of use.

It is recommended that the total volume needed for the analysis is estimated and only this amount is produced.

4.6 Ammonium carbonate, $w[(NH_4)_2CO_3] \geq 99{,}999$ %, for production of mobile phase solution.

4.7 Aqueous ammonia, $w[NH_3(aq.)] \geq 25$ %, for adjustment of pH in the mobile phase.

4.8 Methanol, (CH_3OH), HPLC grade, for production of mobile phase.

4.9 Mobile phase, e.g. 50 mmol/l ammonium carbonate in 3 % methanol at pH 10,3.

Dissolve e.g. 4,80 g of ammonium carbonate (4.6) in approximately 800 ml of water. Adjust the pH to 10,3 with aqueous ammonia (4.7), add 30 ml of methanol (4.8) and fill up to 1 000 ml with water. Filter the mobile phase solution through a 0,45 µm filter prior to use.

The optimal concentration of ammonium carbonate in the mobile phase depends on the analytical column used (e.g. brand, particle size and dimensions). The appropriate concentration of ammonia carbonate is at the discretion of the analyst and should fulfil the criteria for sufficient resolution of the arsenate peak as stated in 5.10.

Methanol is added to the mobile phase in order to enhance the signal intensity for arsenic (carbon enhancement effect [2]). The concentration of methanol for maximum signal depends on the instrument used and should be identified by the analyst.

4.10 Diarsenic trioxide, $w(As_2O_3) \geq 99{,}5$ %, optional.

4.11 Potassium hydroxide solution, $\rho(KOH) = 20$ g/100 ml, optional.

4.12 Sulfuric acid solutions, $w(H_2SO_4) = 20$ % and $w(H_2SO_4) = 1$ %, optional.

4.13 Phenolphthalein, optional.

4.14 Standard solutions, with an arsenic mass concentration of 1 000 mg/l.

The use of commercial standards of arsenic, arsenic III and/or V, with a mass concentration of 1 000 mg/l is recommended.

Otherwise proceed as follows: Dissolve e.g. 1,320 g of diarsenic trioxide (4.10) in 25 ml of potassium hydroxide solution (4.11), neutralize with 20 % sulfuric acid solution (4.12) with phenolphthalein (4.13) as indicator and dilute to 1 000 ml in a volumetric flask with 1 % sulfuric acid solution (4.12).

NOTE By preparing the standard in the extraction solution 1 (4.4) all arsenite will be completely oxidized to arsenate.

4.15 Calibration solutions.

Prepare a range of standards including a blank calibration solution that covers the linear range of the analyte to be determined by diluting the analyte stock solution with extraction solution 1 (4.4). Appropriate matrix matching of the calibration solutions shall be performed by using the extraction solution 1 (4.4) for the final dilution step, which furthermore will prevent reduction of arsenate to arsenite. Transfer an aliquot of the calibration solutions to HPLC vials prior to analysis (6.3.2).

The quantitative oxidation of arsenite to arsenate in the standard solutions should be verified (e.g. visual inspection of chromatogram by looking for an additional peak or a reduced intensity of the arsenate peak).

4.16 Solution for checking chromatographic separation, containing the organic arsenic compounds (e.g. 10 µg/l) monomethylarsenous acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AB), as well as arsenate (e.g. 10 µg/l) and chloride (e.g. 100 mg/l).

5 Apparatus and equipment

5.1 General

To minimize the contamination, all apparatus and equipment that come into direct contact with the sample and the solutions shall be carefully pre-treated. It is recommended to avoid the use of glassware, since this may cause contamination with arsenate, see [3].

WARNING — Some autosampler systems use syringes made of glass. In this case, it is only possible to check for contamination and to minimize it.

5.2 Laboratory grinder, capable of grinding to a particle size of less than 0,5 mm.

5.3 Analytical balance, accuracy of 1 mg.

5.4 Filtering device, for filtration of mobile phase, maximum pore size 0,45 µm.

5.5 Waterbath, adjustable to 90 °C.

5.6 Centrifuge, minimum 2 010 *g* (4 000 min^{-1}).

5.7 Single use syringe filters (0,45 µm) or HPLC vials with filters, compatible with acidic solutions for filtering of test solutions prior to analysis.

5.8 Plastic volumetric flasks, for preparation of mobile phase, blank and calibration solutions.

NOTE If calibration standards are prepared by weighing, plasticware without marks can be used.

5.9 High Performance Liquid Chromatographic System (HPLC).

5.10 Strong anion exchange column (SAX), suitable for selective separation of arsenate from other arsenic compounds present in the sample extracts.

It is advisable to use a guard column to prolong the life-time of the analytical column.

Usually, the minimum acceptable retention time for the analyte is twice the retention time corresponding to the void volume of the column. Furthermore the nearest peak in the chromatogram should be separated from the analyte peak by at least one full peak width at 10 % of the analyte peak height. It is recommended to verify sufficient separation of the analyte peak using a solution of organic arsenic compounds (e.g. monomethylarsenous acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AB)) and arsenate. Make sure that the HPLC run is long enough for chloride (m/z 35) and for arsenic compounds with longer retention times than arsenate, to elute from the column prior to injection of the next sample. It should furthermore be ensured that the arsenate and chloride peaks do not co-elute in order to avoid interference from the polyatomic ion $^{40}Ar^{35}Cl^{+}$ in the mass spectrometer. Examples of chromatographic SAX columns used in the collaborative trial can be found in Annex B, see also [1].

5.11 Inductively coupled plasma mass spectrometer (ICP-MS).

5.12 Argon gas, purity ≥ 99,99 %.

6 Procedure

6.1 General

Follow the procedures for sample preparation as given in EN 13804.

6.2 Waterbath extraction

Weigh a test portion of approximately 0,2 g to 0,5 g sample in powder form to the nearest milligram into a tube and fill up to 10,00 ml with extraction solution 1 (4.4). Include also a reagent blank sample. The tubes shall be securely closed with a tight lid. Shake the tubes thoroughly in order to wet the sample sufficiently with the extraction solution 1 (4.4). Some finely powdered samples may need extended wetting time (e.g. overnight).

If a fresh sample is extracted, the water content shall be taken into account. The sample weight should correspond to 0,2 g to 0,5 g dry matter. The concentration of extraction solution should be adjusted accordingly, keeping the matrix matching at the same level. Proceed e.g. as follows: weigh in the test sample, add water up to 5 ml, mix thoroughly, add 5 ml of extraction solution 2 (4.5) and mix again.

The solutions are then placed in a heated waterbath at 90 °C ± 2 °C and extracted for 60 min ± 5 min.

Following the waterbath extraction step, let the samples be cooled to room temperature and subsequently centrifuge the tubes (10 min, 4 000 min^{-1} (2 010 g)). The supernatant, when transferred to a clean container, can usually be stored in a refrigerator (at approximately 4 °C) for a maximum of one week until analysis (6.3.3). Prior to analysis, all sample extracts should be filtered (5.7) into HPLC vials.

6.3 Determination of inorganic arsenic by HPLC-ICP-MS

6.3.1 Preparation of HPLC-ICP-MS for analysis

The HPLC and ICP-MS operating conditions shall be based on the general information provided by the manufacturer of the instruments taking into account the operating conditions of the analytical column.

An arsenic solution, e.g. 10 µg/l arsenate in 3 % methanol, may be used to optimize the test system according to the manufacturer's instructions. Arsenic is mono-isotopic and can be evaluated at a mass/charge ratio (m/z) of 75.

It is advisable to allow the HPLC system (incl. the analytical column) to equilibrate and ensure stable conditions by turning on the HPLC mobile phase flow in advance prior to start of the analysis. Repeated injections of a sample extract may be necessary until stable chromatography is achieved and the

analytical sequence can be started. Depending on the matrix and column condition, retention time shift can occur and should be taken into account.

6.3.2 Calibration

Inject an appropriate volume of the arsenic calibration solutions (4.15) into an anion exchange HPLC-ICP-MS system and determine the peak area of each of the calibration points to construct a calibration curve.

6.3.3 Determination of samples and blank solution

Inject an appropriate volume of the reagent blank solution and the sample test solutions (6.2) into the anion exchange HPLC-ICP-MS and determine the peak areas under appropriate HPLC-ICP-MS settings, e.g. such as listed in Table 1. Test solutions, which give a response outside the linear calibration range, should be diluted appropriately with extraction solution 1 (4.4) to give a response within the linear calibration range. If a significant blank value occurs, identify the source of this blank. The source should be eliminated and the analysis repeated. If the blank is constant and not avoidable, it should be subtracted. See 7.3.

6.3.4 HPLC sequence

Take measures to control the stability of the instrument sensitivity during the analytical run. Control the instrument sensitivity by e.g. analysing a calibration standard solution throughout the sequence (for example, after each five to 10 samples) and, if necessary, use the results for re-calibration of the system. Another possibility is to introduce an internal standard (e.g. Germanium) post-column (by e.g. a T-split) and use the signal for correction of instrument drift (if any) during the analytical run.

6.3.5 Typical HPLC-ICP-MS settings

Table 1 lists an example of typical settings of HPLC-ICP-MS instrumentation, see also [4].

Table 1 — Example of typical settings of HPLC-ICP-MS instrumentation

Parameter	**ICPMS settings**
ICP-MS	Agilent 7 500ce
RF power (W)	1 500
Carrier gas flow (l min^{-1})	1,2
Plasma gas flow (l min^{-1})	15
Auxiliary gas flow (l min^{-1})	1,0
Mass resolution (amu)	0,6 to 0,8
Integration time (ms)	1000
Isotopes monitored (m/z)	75 (As), 35 (Cl)
Parameter	**HPLC settings**
HPLC	Agilent 1 100
Column	IonPac AS7 (250 mm × 2 mm)
Flow rate (ml min^{-1})	0,15
Column temperature	Room temperature
Operating pressure (bar)	50
Injection volume (µl)	5
Autosampler temperature	Room temperature
Measurement time (min)	15

6.4 Quality control

As an analytical control, reference samples having reliable known inorganic arsenic contents shall be analysed in parallel with all the series of samples to estimate the trueness. The reference samples are to be subjected to all the steps in the method starting from waterbath extraction. This also applies to the preparation of blank solution.

If reference samples are not available, spike experiments should be performed and the recovery used to estimate trueness of the analysis. It is advisable to check for memory effects, e.g. by analysis of blank solutions after reference materials.

The oxidation of arsenite to arsenate should be verified by performing recovery experiments from spiking of a known amount of arsenite to a test sample in the extraction step. If this oxidation is not complete the amount of sample shall be reduced and/or the hydrogen peroxide solution (4.3) shall be checked for degradation.

7 Calculation

7.1 Integration of peaks

The retention time of arsenate is identified from the analysis of the calibration solutions. The arsenate peak area in the standards, reagent blank and sample extract solutions is determined.

7.2 Inorganic arsenic in test solutions

Calculate the concentration of inorganic arsenic in the test solutions using the calibration function established by linear regression from the calibration curve.

7.3 Calculation of inorganic arsenic in the samples

Calculate the mass fraction w of inorganic arsenic in milligram per kilogram sample according to Formula (1):

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

where

- ρ is the mass concentration of inorganic arsenic in the sample test solution, in microgram per litre;
- V is the volume of extraction solution for waterbath extraction (usually 0,01 l), in litre;
- m is the mass of test portion, in gram;
- F is the dilution factor.

If the sample is dried prior to analysis, the result should be corrected for the moisture content.

The mass concentration of inorganic arsenic in the blank solution shall be as low as possible (see 6.3.3). If the inorganic arsenic concentration in the blank is constant and not avoidable, it should be subtracted from ρ.

8 Precision

8.1 General

Results from an interlaboratory test are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given in Annex A. Further information can be found in a report on the conduction and results from the interlaboratory test [1].

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 2 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 2 in not more than 5 % of the cases.

Table 2 — Mean values, repeatability (*r*) and reproducibility (*R*) limits for inorganic arsenic in foodstuffs included in the collaborative test

Foodstuff	$\bar{x}$ mg/kg	*r* mg/kg	*R* mg/kg
White rice	0,073	0,010	0,022
Wholemeal rice	0,47	0,03	0,12
Leek	0,086	0,015	0,033
Blue mussels	0,33	0,06	0,14
Fish muscle	0,27	0,05	0,11
Seaweed	10,3	1,2	3,4

9 Test report

The test report should fulfil the requirements in EN ISO IEC 17025 [4] and specify at least the following:

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

b) the test method used and the elemental species to be determined, with reference to this European Standard;

c) the test results obtained and the units in which they are specified;

d) the date of sampling and sampling procedure (if known);

e) the date when the analysis was finished;

f) 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)

Precision data

The precision and trueness of the method was established by the CEN TC 275 "Food analysis – Horizontal methods" Working Group 10 "Elements and their chemical species" in an interlaboratory test among 13 laboratories performed in 2013 under the mandate given by the EU Commission, see Table A.1 according to ISO 5725-2 [5]. The participants analysed six different foodstuff samples in duplicates. Further details on the study can be found in the report from the interlaboratory test [1].

Table A.1 — Precision data

Sample	White rice	Wholemeal rice	Leek	Blue mussels	Fish muscle	Seaweed
Number of laboratories	13	13	13	13	13	13
No laboratories after elimination of outliers	13	12	12	13	13	13
No outlier laboratories	0	1	1	0	0	0
Mean value $\bar{x}$, mg/kg	0,073	0,47	0,086	0,33	0,27	10,3
Repeatability limit r, mg/kg	0,010	0,025	0,015	0,057	0,049	1,2
Repeatability standard deviation s_r, mg/kg	0,0036	0,0090	0,0054	0,020	0,017	0,44
RSD(r), %	4,9	1,9	6,3	6,2	6,3	4,3
Reproducibility limit R, mg/kg	0,022	0,12	0,033	0,14	0,11	3,4
Reproducibility standard deviation s_R, mg/kg	0,008	0,043	0,012	0,049	0,038	1,2
RSD(R), %	11,0	9,1	13,8	14,9	13,8	11,8
Horwitz value according to Horwitz [6]	23,54	17,79	22,95	18,79	19,29	11,20
HorRat value according to Horwitz [6]	0,47	0,51	0,60	0,79	0,72	1,05
Horwitz value according to Thompson [7]	22,00	17,92	22,00	18,93	19,43	11,26
HorRat value according to Thompson [7]	0,50	0,51	0,63	0,79	0,71	1,04

Annex B
(informative)

Supplementary information about chromatographic conditions

The following strong anion exchange (SAX) columns were used by the participants in the collaborative trial:

— IonPac AS7

— ICSep Ion120

— Hamilton PRP-X100

It is recommended to use a guard column to protect and prolong the life-time of the analytical column. The instructions from the column producers should be followed when selecting the chromatographic conditions for the column e.g. temperature, mobile phase flow, injection volume.

The participants in the collaborative trial used typically a mobile phase concentration in the range of 20 mmol/l to 50 mmol/l to achieve a satisfactory separation of the arsenate peak from the peaks of other arsenic compounds.

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