SLOVENSKI STANDARD
oSIST prEN 17266:2018

01-oktober-2018

Živila - Določevanje elementov in njihovih kemijskih oblik - Določevanje živosrebrovih organskih spojin v morskih sadežih z analizo elementarnega živega srebra

Foodstuffs - Determination elements and their chemical species - Determination of organomercury in seafood by elemental mercury analysis

Lebensmittel - Bestimmung von Elementen und ihren Verbindungen - Bestimmung von Organoquecksilber in Fisch- und Meeresfrüchten mit Feststoffquecksilberbestimmung

Produits alimentaires - Dosage des éléments et de leurs espèces chimiques - Dosage du mercure organique dans les fruits de mer par analyse du mercure élémentaire

Ta slovenski standard je istoveten z: **prEN 17266**

ICS:

67.120.30	Ribe in ribji proizvodi	Fish and fishery products

oSIST prEN 17266:2018 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 17266

July 2018

ICS 67.120.30

English Version

Foodstuffs - Determination elements and their chemical species - Determination of organomercury in seafood by elemental mercury analysis

Produits alimentaires - Dosage des éléments et de leurs espèces chimiques - Dosage du mercure organique dans les fruits de mer par analyse du mercure élémentaire

Lebensmittel - Bestimmung von Elementen und ihren Verbindungen - Bestimmung von Organoquecksilber in Fisch- und Meeresfrüchten mit Feststoffquecksilberbestimmung

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Ref. No. prEN 17266:2018 E

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European foreword

This document (prEN 17266:2018) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

1 Scope

This document describes a method for the determination of organomercury in seafood/fishery products by elemental mercury analysis. The method has been successfully valideted in an interlaboratory study with a working range from 0,013 mg/kg to 5,12 mg/kg (HORRAT values < 2) in seafood/fishery products [1], [2]. The limit of quantification is approximately 0,010 mg/kg organomercury (referring to dry weight, expressed as mercury) [3], [4].

Organic species of mercury, other than monomethylmercury, are also extracted and thus determined with this method. However, in seafood/fishery products the contribution from organic species of mercury other than monomethylmercury is negligible.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 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 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

4 Principle

Organomercury in seafood/fishery products is separated from the matrix by double liquid-liquid extraction, first with an organic solvent (toluene) and subsequently with L-cysteine solution and is detected and determined by elemental mercury analyser [3], [4].

Elemental mercury analyser, also known as automated or direct mercury analyser, is a single purpose atomic absorption spectrophotometer for mercury determination. The determination of mercury with an elemental mercury analyser is based on sample drying and subsequent thermal decomposition, followed by electro thermal atomisation of mercury. A gold amalgamator selectively traps and pre-concentrates the mercury from the flow of decomposition products. Finally, the trapped mercury is thermally released and detected by atomic absorption at 253,7 nm. Organomercury results are expressed in mg/kg as mercury.

The sample solution can be detected with alternative detection techniques provided that equivalence of method performance is proven.

5 Reagents

The mass concentration of mercury in the reagents and water used shall be low enough not to affect the results. All reagents shall be of analytical grade, i.e. pro analysi, p.a. or similar unless otherwise specified.

Use water conforming to grade 2 of EN ISO 3696.

5.1 Nitric acid, minimum w = 65 %[1)], density about 1,4 g/ml

5.2 Hydrochloric acid, minimum w = 32 %, p. a., ρ = 1,18 g/ml[2)]

5.3 Diluted hydrochloric acid solution,

Mix equal volumes of hydrochloric acid (5.2) and water.

5.4 Hydrobromic acid, w = 47 %, p. a., ρ = 1,47 g/ml

5.5 Toluene, p. a.

5.6 L-cysteine monohydrate hydrochloride, Ph. Eur. or USP grade [3)]

5.7 Sodium sulfate anhydrous, p. a.

5.8 Sodium acetate anhydrous

5.9 L-cysteine solution, ρ = 1 g/100 ml

Weigh (6.3) 1,0 g of L-cysteine monohydrate hydrochloride, 1,25 g of sodium sulfate (5.7) and 0,8 g of sodium acetate (5.8) into a 100 ml beaker. Add about 75 ml of water and stir until complete dissolution. Transfer this solution completely to a 100 ml volumetric flask and make up to volume with water. This solution can be stored for 1 day at ambient temperature. Other preparation volumes may be used as long as proportions are kept.

The mass concentration of mercury in the L-cysteine solution should be as low as possible. The purity of this solution should be such that the response for mercury shall be less than half the response of the 1 µg/l mercury standard solution (calibration solution 9, see 5.13.1).

5.10 Mercury reference solution, of 1 000 mg/l Hg

5.11 Monomethylmercury (MMHg) chloride, minimum purity of 95 %

5.12 Mercury stock solutions

5.12.1 Stock solution 1 (ρ = 10 mg/l Hg)

Pipette 1,00 ml of the commercial mercury reference solution 1 000 mg/l Hg (5.10) in a 100 ml volumetric flask; add 2 ml of diluted hydrochloric acid solution (5.3) and make up to volume with water. This solution is stable in a glass container in the refrigerator at approximately 2 °C to 10 °C for 6 months.

5.12.2 Stock solution 2 (ρ = 500 µg/l Hg)

Pipette 2,50 ml of stock solution 1 (5.12.1) in a 50 ml volumetric flask, add 1 ml of diluted hydrochloric acid solution (5.3) and make up to volume with water. This solution is stable in a glass container in the refrigerator at approximately 2 °C to 10 °C for 2 months.

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1) w = mass fraction.

2) ρ = mass concentration.

3) Ph. Eur. = European Pharmacopoeia; USP = United Stated Pharmacopoeia.

5.13 Calibration solutions

5.13.1 Mercury calibration solutions

Due to the highly stable response of elemental mercury analysers, there is no need to recalibrate for each analytical sequence. Calibration is usually stable for at least 1 year. For that reason every instrumental calibration will be maintained for that period, provided that quality controls for each sequence are satisfactory. Nevertheless, if the gold amalgamator or catalyst tube is changed, response could change and a new calibration is necessary . In such a case, analyse 500 µl of each calibration solution (from blank to 100 µg/l Hg). for selection of corresponding standard solutions for each calibration curve, see 6.4. or acceptance criteria regarding the calibration curve, see 7.3.

Select calibration standard solutions depending on the cell used, thus on the expected concentration level of the sample. This is comprehensively described in 6.4.

Prepare all calibration standard solutions freshly for each calibration. Other volumes of preparation are suitable provided that they maintain the proportions described below.

Prepare calibration standard solutions from either the stock solution 2 (5.12.2) or the calibration solution 3 (see Table 1), placed into a 50 ml volumetric flask and filled up to the mark with L-cysteine solution (5.9) according to the scheme presented in Table 1.

Table 1 — Example of mercury calibration standard solutions

Calibration standard solution no	Initial solution	Quantity of initial solution ml	Final Hg concentration (ρ) µg/l
1	stock solution 2 (5.12.2)	10	100
2	stock solution 2 (5.12.2)	7,5	75
3	stock solution 2 (5.12.2)	5,0	50
4	stock solution 2 (5.12.2)	2,5	25
5	stock solution 2 (5.12.2)	1,5	15
6	stock solution 2 (5.12.2)	1,0	10
7	stock solution 2 (5.12.2)	0,5	5,0
8	calibration solution no 3	2,5	2,5
9	calibration solution no 3	1,0	1,0

5.13.2 Blank solution for calibration

Use L-cysteine 1 % (w/v) solution (5.9) as blank (level 0) for instrumental calibration.

5.14 Internal quality control solutions

5.14.1 General

As the response of elemental mercury analysers is highly stable, there is no need to recalibrate the instrument for each analytical sequence. However, some control solutions are used to ensure the validity of the latest calibration. Each calibration curve needs to be compared against an external solution to demonstrate absence of error in intermediate standard solutions preparation.

Quality control solution 2 (QC2) ensures that quantification at low level is still correct. Quality control solution 1 (QC1) demonstrates that the response is stable at high concentrations and that no uncontrolled drifts is detected, since QC1 is placed at the end of the analytical sequence.

As an alternative the analysis of a certified reference material may be used.

5.14.2 Quality control solution 1 (QC1), (ρ = 50 µg/l Hg)

Pipette 5,0 ml of stock solution 2 (5.12.2) in a 50 ml volumetric flask and make up to volume with the L-cysteine solution (5.9). Prepare freshly for each analytical sequence.

The following alternative options for the preparation of QC1 are provided as examples:

— QC1 may be prepared, using intermediate solutions prepared from brands or batches of the commercial 1 000 mg/l Hg (5.10) reference solutions different than those used for the preparation of the calibration standard solutions.

— QC1 may be prepared from a standard solution of MMHg (5.11). In that case, pipette 2,7 ml of spiking solution 2 (5.15.2) in a 50 ml volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.9).

5.14.3 Quality control solution 2 (QC2), (ρ = 1 µg/l Hg)

Pipette 1,0 ml of QC1 (5.14.2) in a 50 ml volumetric flask and make up to volume with the L-cysteine solution (5.9). Prepare freshly for each analytical sequence.

5.15 Spiking Solutions

5.15.1 Spiking solution 1 (ρ = 76,7 mg/l, expressed as Hg)

Weigh accurately 24 mg of monomethylmercury chloride (5.11, taking into account the purity for the final concentration), in a 250 ml volumetric flask, add about 4 ml of diluted hydrochloric acid solution (5.3) and 200 ml of water. Shake thoroughly until complete solubilisation and make up to volume with water. This solution is stable in a glass container in a refrigerator (at approximately 2 °C to 10 °C) for 1 year.

5.15.2 Spiking solution 2 (ρ = 0,96 mg/l, expressed as Hg)

Pipette 625 µl of spiking solution 1 (5.15.1) in a 50 ml volumetric flask, add 1 ml of diluted hydrochloric acid solution (5.3) and make up to volume with water. This solution is stable in a glass container in a refrigerator (at approximately 2 °C to 10 °C) for 3 months.

6 Apparatus and equipment

All equipment and labware that come into direct contact with the sample and the solutions used shall be carefully pretreated/cleaned corresponding to EN 13804 to minimize the blank value.

6.1 Elemental mercury analyser.

6.2 Consumables for the equipment used, e.g. cells for 0,5 ml.

6.3 Analytical balance, accuracy of at least 1 mg.

6.4 Centrifuge, capable of 3 600 *g.*

6.5 Centrifuge tubes, made of polypropylene or PTFE.

6.6 Vial for chromatography, glass vial with cap.

7 Procedure

7.1 Sample preparation

Weigh accurately 0,7 g to 0,8 g of sample (or 0,2 g in the case of lyophilised samples, plus 0,5 ml of water) in a 50 ml centrifuge tube, add 10 ml of hydrobromic acid (5.4) and shake it manually for at least 2 min, then add 20 ml of toluene (5.5) and shake it vigorously (e.g. vortex) for at least 2 min. Centrifuge for 10 min at 2 300 *g*. Transfer about 15 ml from the organic supernatant into a 50 ml centrifuge tube containing already 6,0 ml of L-cysteine solution (5.9).

Add additional 15 ml of toluene to the initial centrifuge tube (containing the hydrobromic acid phase), and repeat a second extraction with the organic phase. After centrifugation, transfer the remaining upper organic phase into the 50 ml centrifuge tube with the L-cysteine solution (5.9). Shake it vigorously (e.g. vortex) for at least 2 min and centrifuge for 10 min at 2 300 *g*. Take an aliquot of 2 ml to 3 ml from the lower phase with the L-cysteine (and the extracted organic mercury) and place it into a vial for chromatography (6.6). Ensure that the sample to be analysed is toluene free. Test samples should be analysed as soon as possible to minimize instability issues.

In case an emulsion is formed at the interface between the organic toluene phase and the hydrobromic acid phase, tap the container a few times against the table and centrifuge again at a higher speed (about 3 600 *g* for 10 min). After 2 min the two phases should be completely separated. If interphase does not desapear, leave it overnight to improve the separation of phases.

Sample test solutions with Hg concentrations exceeding the highest calibration level should be appropriately diluted with L-cysteine solution (5.9) or reanalysed injecting into the instrument less volume of sample extract to ensure measurement within the calibration range. If the sample is suspected to have higher Hg concentration (for predators as tuna or shark) lower amount of sample should be used for the extraction.

7.2 Reagent blank solution

Parallel to the extraction of the samples, analyse the reagent blank solution not including the sample applying the procedure described in 6.1.

7.3 Determination by elemental mercury analyser

Switch on the instrument and let it reach the working temperatures. “Clean” the system (some software have a “clean” option which raises the temperatures of catalyser and amalgamator for a determined time). Subsequently place about 0,5 ml of water in the cell and perform the analysis. Finally, perform a background control with an empty cell. The result should be ≤ 0,3 µg/l Hg. If not, clean the system again as described before. The analytical sequence (7.5) or calibration (5.13, 7.4) should be performed 2 min to 3 min after the cleaning step.

7.4 Instrumental parameters

The following parameters are provided as an example; they may be adapted depending on the instrument used:

Drying time:	250 s
Drying temperature:	(285 ± 25) °C
Decomposition time:	150 s
Decomposition temperature:	(725 ± 25) °C
Volume of analysis (for sample solutions, standard solutions and blanks):	500 µl