



SLOVENSKI STANDARD

SIST EN 16801:2016

01-junij-2016

Živila - Določevanje elementov in njihovih kemijskih oblik - Določevanje metil živega srebra v živilih morskega izvora z izotopnim razredčenjem GC-ICP-MS

Foodstuffs - Determination of elements and their chemical species - Determination of methylmercury in foodstuffs of marine origin by isotope dilution GC-ICP-MS

Lebensmittel - Bestimmung von Elementen und ihren Verbindungen - Bestimmung von Methylquecksilber in Lebensmitteln marinen Ursprungs mit Isotopenverdünnung GC-ICP-MS

(standards.iteh.ai)

Produits alimentaires - Détermination des éléments et de leurs espèces chimiques - Détermination de la teneur en méthylmercure dans les produits alimentaires d'origine marine par dilution isotopique GC-ICP-MS

Ta slovenski standard je istoveten z: EN 16801:2016

ICS:

67.120.30 Ribe in ribji proizvodi Fish and fishery products

SIST EN 16801:2016

en,fr,de

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST EN 16801:2016

<https://standards.iteh.ai/catalog/standards/sist/9d5e80b7-069b-4261-bbbb-e51bf6f81acd/sist-en-16801-2016>

EUROPEAN STANDARD

EN 16801

NORME EUROPÉENNE

EUROPÄISCHE NORM

March 2016

ICS 67.120.30

English Version

Foodstuffs - Determination of elements and their chemical species - Determination of methylmercury in foodstuffs of marine origin by isotope dilution GC-ICP-MS

Produits alimentaires - Détermination des éléments et de leurs espèces chimiques - Détermination de la teneur en méthylmercure dans les produits alimentaires d'origine marine par dilution isotopique GC-ICP-SM

Lebensmittel - Bestimmung von Elementen und ihren Verbindungen - Bestimmung von Methylquecksilber in Lebensmitteln marinen Ursprungs mit Isotopenverdünnung GC-ICP-MS

This European Standard was approved by CEN on 8 February 2016.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

(standards.iteh.ai)

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

<https://standards.iteh.ai/catalog/standards/sist/9d5e80b7-069b-4261->

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents		Page
European foreword.....		3
1	Scope.....	4
2	Normative references.....	4
3	Principle.....	4
4	Reagents.....	4
5	Apparatus and equipment.....	6
6	Procedure.....	7
7	Calculation.....	10
8	Precision.....	11
9	Test report.....	12
Annex A (informative) Precision data		13
Bibliography.....		14

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST EN 16801:2016

<https://standards.iteh.ai/catalog/standards/sist/9d5e80b7-069b-4261-bbbb-e51bf6f81acd/sist-en-16801-2016>

European foreword

This document (EN 16801:2016) 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under mandate M 422 given to CEN by the European Commission and the European Free Trade Association.

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

PREVIEW
(standards.iteh.ai)

[SIST EN 16801:2016](https://standards.iteh.ai/catalog/standards/sist/9d5e80b7-069b-4261-bbbb-e51bf6f81acd/sist-en-16801-2016)

<https://standards.iteh.ai/catalog/standards/sist/9d5e80b7-069b-4261-bbbb-e51bf6f81acd/sist-en-16801-2016>

1 Scope

This European Standard describes a method for the determination of monomethylmercury (MMHg) in foodstuffs of marine origin. The method has been validated in an interlaboratory test on mussel tissue, squid muscle, crab claw muscle, dog fish liver, whale meat, cod muscle and Greenland halibut muscle (all freeze-dried) with mass fractions from 0,04 mg/kg to 3,6 mg/kg dry weight according to ISO 5725-2 [1].

Laboratory experiences have shown that this method is also applicable on fresh samples [2].

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

The sample is spiked with an appropriate amount of Hg-isotope enriched MMHg and digested using tetramethylammonium hydroxide (TMAH). After pH adjustment, derivatisation and extraction, the organic phase is analysed using GC-ICP-MS. The GC separates the different mercury species before the derivatised species (ethylmethylmercury) is atomised and ionised in the high temperature by the ICP. The ions are extracted from the plasma by a set of sampler and skimmer cones and transferred to a mass spectrometer where the ions are separated by their mass/charge ratio and determined by a pulse-count and/or analogue detector. The result is calculated using the isotope dilution equation.

WARNING — The use of this method may involve hazardous materials, operations and equipment. This method does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

4 Reagents

4.1 General

The concentration of mercury 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 $\mu\text{S}/\text{cm}$ at 25 °C) and acid of high purity is recommended, e.g. cleaned by sub-boiling distillation. 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 Monomethylmercury stock solutions.

Commercially available MMHg standard enriched in the ^{201}Hg -isotope with a mass fraction of $5,5 \mu\text{g/g}$ (as Hg) is recommended, such as IES-MMHg201¹⁾. Other MMHg Hg-isotope enriched standards may also be available in suitable mass fractions from other suppliers or may be prepared in-house. In this case, the method shall be adjusted accordingly. The quality of the standards should be designed to be used by isotope dilution methods. Stock solutions in diluted acid are preferred.

4.3 Monomethylmercury standard solution.

4.3.1 General

The mass fractions of the MMHg in the standard solutions shall be chosen in relation to the expected mass fraction of MMHg in samples. It is important that all dilutions are done by weighing so that their accurate mass fractions can be calculated. The following descriptions are given as examples.

4.3.2 MMHg approximately 500 ng/g (as Hg).

Dilute approximately 1 g, to the nearest milligram, of ^{201}Hg enriched MMHg stock solution (4.2) with water up to 10 g. Calculate the exact mass fraction using the mass fraction of the stock solution and weight.

4.3.3 MMHg approximately 50 ng/g (as Hg).

Dilute approximately 1 g, to the nearest milligram, of the 500 ng/g ^{201}Hg enriched MMHg solution (4.3.2) with water up to 10 g. Calculate the exact mass fraction using the exact mass fraction of the 500 ng/g solution and weights. (standards.iteh.ai)

4.3.4 MMHg approximately 5 ng/g (as Hg).

Dilute approximately 1 g, to the nearest milligram, of the 50 ng/g ^{201}Hg enriched MMHg solution (4.3.3) with water up to 10 g. Calculate the exact mass fraction using the exact mass fraction of the 50 ng/g solution and weights.

4.4 Tetramethylammonium hydroxide (TMAH), mass fraction $w = 25 \%$ in water, minimum synthesis quality.

4.5 Acetic acid, concentrated, mass concentration $\rho = 1,05 \text{ g/ml}$, minimum p.a. quality.

4.6 Sodium hydroxide, minimum p.a. quality.

4.7 Sodium hydroxide solution, substance concentration $c(\text{NaOH}) = 0,1 \text{ mol/l}$.

Transfer 0,4 g of sodium hydroxide to a 100 ml volumetric flask and add water to the mark.

4.8 Sodium acetate, minimum p.a. quality.

4.9 Sodium acetate/acetic acid buffer (pH 5).

Dissolve 41 g of sodium acetate in approximately 0,5 l of water. Adjust the pH of the solution to 5 by adding concentrated acetic acid (4.5) dropwise by using a pH-meter (5.4). Finally, dilute the solution to 1 l with water.

¹⁾ IES-MMHg201 is available from e.g. Innovative Solutions in Chemistry S.L., Edificio Científico-Tecnológico, Campus de "El Cristo", 33006, Oviedo, Spain <http://www.isc-science.com/> or Qmx Laboratories, bolford Street, Thaxted, Essex, CM6 2PY, UK <http://www.qmx.com/>. This is an example of a suitable product available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by CEN of this product.

EN 16801:2016 (E)

4.10 Nitric acid, 65 %, ρ of approximately 1,4 g/ml. Hg-free quality. Other acid concentrations may be used if the volume added in 6.2 is adjusted accordingly.

4.11 Sodium tetraethyl borate, minimum synthesis quality (98 %).

4.12 Sodium tetraethyl borate solution (2 %).

Dissolve 1 g of sodium tetraethyl borate in 0,1 mol/l of sodium hydroxide solution (4.7), transfer to a 50 ml-volumetric flask and fill up to the mark with 0,1 mol/l sodium hydroxide solution. Prepare freshly at each day of analysis or divide the solution into smaller amounts and store in the freezer at approximately $-20\text{ }^{\circ}\text{C}$. The solution may be stored at approximately $-20\text{ }^{\circ}\text{C}$ for at least three months. The solution shall be used within the day after removal from the freezer.

4.13 Hexane, minimum HPLC-quality.

4.14 Optimising solution for the ICP-MS.

The optimising solution should contain elements that cover the whole mass range giving a high rate of oxides and doubly charged ions. Use the solutions recommended by the manufacturer of the ICP-MS instrument. A solution containing e.g. Li, Ce and Tl is suitable for those purposes. In this case, choose the concentration of these elements in order to achieve a count rate of $> 10\ 000$ cps (counts per second).

5 Apparatus and equipment

All pieces of equipment described here are examples of suitable equipment and may be replaced by equivalent equipment unless otherwise stated. Generally, clean and rinse the vessels carefully according to the procedure in EN 13804. In addition to standard laboratory equipment, use the following:

5.1 Analytical balance, accuracy of 0,5 mg. [SIST EN 16801:2016
https://standards.iteh.ai/catalog/standards/sist/9d5e80b7-069b-4261-](https://standards.iteh.ai/catalog/standards/sist/9d5e80b7-069b-4261-bb1a-511881a71c00/en-16801-2016)

5.2 Orbital/overhead rotator, capable of approximately 0,04 g (20 min^{-1}).

5.3 Centrifuge, capable of 1 200 g ($4\ 000\text{ min}^{-1}$).

5.4 pH-meter.

5.5 Laboratory ware, volumetric flasks of glass, polypropylene tubes (10 ml) for samples, GC-vials, pH paper.

5.6 Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Mass spectrometer with inductively coupled argon plasma operating in a mass range from 5 amu (atomic mass units) to 240 amu. Using routine settings the mass spectrometer shall be capable to resolve 1 amu peak width at 5 % peak height or better (resolution 300) with sufficient sensitivity to achieve the detection limits suitable for the analytical purpose.

5.7 Argon, purity $\geq 99,99\%$.

5.8 Gas chromatograph (GC), with injector heating, programmable column heating and heating of transfer line to ICP-MS.

5.9 GC-column, capillary or preparative column capable of separating ethylmethylmercury from other mercury species (e.g. 30 m x 0,32 mm, analytical column with 5 % phenyl methyl siloxane; film thickness: 0,25 μm).

5.10 Helium, purity $\geq 99,99\%$.

5.11 Helium, (5.10) with 1 % to 2 % added xenon for tuning of the GC-ICP-MS interface or some other tuning configuration capable of optimising the instrument parameters, optional.

5.12 Oxygen, optional, to prevent carbon deposition, according to manufacturer's instructions, e.g. 5 %.

6 Procedure

6.1 Calculation of optimal spike amount

The following description is given for the ^{201}Hg enriched MMHg spike solution (4.2). To ensure that the measurement is within acceptable error limits, the abundance of the isotopes ^{200}Hg , ^{201}Hg and ^{202}Hg in the spiked sample should be as close as possible [3]. This is achieved when the amount of spike to analyte is 1 to 7 for the given isotope abundances in Table 1.

The ratio of 1 to 7 applies for the specified spike solution with the given isotopic composition. If another spike solution with another isotope composition is used, the factor should be adjusted accordingly.

Table 1 — Isotopic composition of natural Hg and recommended ^{201}Hg enriched spike

Hg-isotope	% of isotope in natural Hg	% of isotope in enriched Hg
200	23,10	0,89
201	13,18	96,50
202	29,86	2,37

Calculate the appropriate spike amount in gram, m_{Sp} , to be added from either 4.3.2, 4.3.3 or 4.3.4 to the sample using Formula (1):

$$m_{\text{Sp}} = \frac{\left(\frac{w_{\text{Se}} \times m_{\text{S}}}{7} \right)}{w_{\text{Sp}}} \quad (1)$$

where

w_{Se} is the estimated mass fraction of MMHg in the sample, in ng/g or $\mu\text{g}/\text{kg}$;

m_{S} is the mass of the sample to be analysed, in g;

7 is the factor needed to achieve approximate abundance matching of the isotopes in the spiked sample;

w_{Sp} is the exact mass fraction from the diluted MMHg stock solutions 4.3.2, 4.3.3 or 4.3.4, in ng/g.

The amount of spike to be added should not be below 0,1 g and should not exceed 1 g. This is to ensure enough significant figures from the weighing of the spike and to ensure the complete dissolution of the sample after TMAH addition, respectively.

EXAMPLE For a sample with an estimated MMHg mass fraction, w_{Se} of 100 ng/g ($\mu\text{g}/\text{kg}$) where the mass of the sample to be analysed, m_{S} , is 0,2 g; m_{Sp} would be approximately 0,006 g for solution 4.3.2, approximately 0,06 g for solution 4.3.3 and approximately 0,6 g for solution 4.3.4. This is an example with rounded numbers, use exact mass fractions for analysis.