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

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National foreword

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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 CG-ICP-SM

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

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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of an identical text or by endorsement, at the latest by September 2016, and conflicting national standards shall be withdrawn at the latest by September 2016.

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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 µS/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 µg/g (as Hg) is recommended, such as IES-MMHg201[1)]. 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.

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 ρ = 1,05 g/ml, minimum p.a. quality.

4.6 Sodium hydroxide, minimum p.a. quality.

4.7 Sodium hydroxide solution, substance concentration c(NaOH) = 0,1 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.

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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.

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 °C. The solution may be stored at approximately −20 °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.

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

5.3 Centrifuge, capable of 1 200 g (4 000 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 ≥ 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 ≥ 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):

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

where

w_{Se} is the estimated mass fraction of MMHg in the sample, in ng/g or µg/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 (µg/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.

If the sample has been analysed for total mercury content, this value can be used as, w_{Se}, the estimated mass fraction of MMHg in the calculation. A correction factor should be used depending on the amount of MMHg of the total Hg (e.g. muscle from fish would give a factor of around 1 to 0,8, whereas fish liver is usually in the range of 0,4 to 0,6 and mussels in the range of 0,2 to 0,6).

6.2 Sample preparation

Weigh a test portion of 0,2 g to the nearest milligram in a polypropylene tube and spike with the calculated amount to the nearest milligram of the appropriate ^{201}Hg enriched MMHg solution (4.3.2, 4.3.3 or 4.3.4). This step is crucial for the correct result. Add 3 ml of TMAH solution (4.4) to the tube and mix the contents by gentle rotating (5.2) until the sample is completely dissolved, if necessary overnight. After digestion, add 1 ml of pH 5 sodium acetate/acetic acid buffer (4.9) followed by 600 µl of nitric acid (4.10) and mix the solution using a swirl mixer. Add 1 ml of hexane (4.13) and then 500 µl of sodium tetraethyl borate (4.12) to derivatise the sample. Cap the tube and mix the contents by rotating for 10 min before centrifugation at 1 200 *g* for 5 min. Transfer the hexane layer to a GC-vial and analyse the sample on the GC-ICP-MS instrument.

When using other nitric acid concentrations than described in 4.10, the volume to be added shall be adjusted accordingly so that a pH of 5 is achieved after acid addition and mixing.

NOTE: When using isotopic dilution, the efficiency of both the derivatisation and extraction into hexane does not need to be taken into account. However, too low a derivatisation/extraction efficiency can limit the sensitivity for samples with low MMHg mass fractions.

Low level species transformation is known to take place during extraction with TMAH, pH adjustment and derivatisation [4]. Verify the trueness of the method by determining reference materials of similar matrix as the samples to be determined.

6.3 ICP-MS conditions

Use the instrument parameters described in the manufacturers' operating manual.

Different ranges of instrument settings used in the interlaboratory test are given in Table 2, see also [5].

Table 2 — Example of instrument settings for ICP-MS

Parameter	Setting
Radiofrequency (RF-)Power (W)	600 to 1 600
Carrier gas flow (l min $^{-1}$)	0,1 to 1,7
Plasma gas flow (l min $^{-1}$)	13 to 16
Make up gas flow (l min $^{-1}$)	0 to 1
Optional gas (oxygen)	5 %
Mass resolution (amu)	0,8
Integration time (ms)	25 to 50
Isotopes measured	^{200}Hg, ^{201}Hg, ^{202}Hg

The integration time should be short enough to provide enough points per chromatographic peak depending on the GC-column used. It is recommended to collect 12 points to 20 points per chromatographic peak in order to define it properly. Shorter or longer integration times on the isotopes may influence the sensitivity and accuracy of the determination.

6.4 GC conditions

Use the instrument parameters described in the manufacturers' operating manual.

An example of instrument settings is given in Table 3.

Table 3 — Example of instrument settings for capillary GC

Parameter	Setting
Injection mode	Splitless or on-column
Injection volume (µl)	0,2 to 3
Injection temperature (°C)	100 to 180
Transfer line temperature (°C)	200 to 250
Carrier gas flow (ml min^{-1})	1,2 to 2,6
GC oven temperature program	$50\ ^{\circ}C(1\,min) \xrightarrow{50\ ^{\circ}C\,min^{-1}} 200\ ^{\circ}C(1\,min) \xrightarrow{50\ ^{\circ}C\,min^{-1}} 280\ ^{\circ}C(1{,}7\,min)$

6.5 Set up procedures for the ICP-MS and GC-ICP-MS

Before starting routine measurements, the following set up procedure should be applied: Warm up the ICP-MS in full running mode for a minimum of 20 min to 30 min.

Use the optimising solution (4.14) to check the mass resolution and mass calibration and to adjust maximum sensitivity at low rates of oxides and doubly charged ions. Use the optimising solution to tune the instruments plasma parameters, ion lenses, analyser parameters and detector parameters.

In the event of high MMHg mass fractions, specific instrumental setups shall be properly adjusted (e. g dead time correction, dual detector calibration) or the samples should be diluted and reanalysed.

Tune the interface after the GC is connected to the ICP-MS. The GC-ICP-MS interface can be tuned using 1 % to 2 % xenon in the carrier gas (helium) for the GC (5.11). The plasma parameters such as: x,y,z position of the torch, carrier gas flow, plasma RF-power and auxiliary gas flow may be tuned using one or more Xe-masses (e.g. ^{124}Xe, ^{129}Xe or ^{132}Xe). If a sufficient sensitivity cannot be achieved simply by tuning the plasma parameters, the ion lens parameters may also be tuned using the same Xe-masses. Other tuning setups (e.g. $^{36}Ar^{40}Ar$ or ^{202}Hg) may also be used provided they are able to produce sufficient sensitivity for the determination.

6.6 Sample measurement

6.6.1 Preparation of the GC-ICP-MS for analysis

Run the sample with the estimated lowest level of MMHg, either a reference material (e.g. NIST SRM 1566b[2], see also [5] or a derivatised standard solution (e.g. 10 µg/l) to check that the instrument sensitivity and the chromatographic separation of the system are satisfactory and fit for purpose.

2) NIST SRM 1566b is an example of a suitable product available commercially. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this product.

6.6.2 Determination of samples and blank solution

Inject appropriate amounts of the instrument blank, reagent blank and samples and determine the peak areas for the ^{200}Hg, ^{201}Hg and ^{202}Hg masses. If a significant blank value for MMHg is identified, the source of this blank should be identified. The source should be eliminated and the analysis repeated.

NOTE By use of isotopic dilution the calibration of the instruments with known amounts of MMHg is not necessary.

6.6.3 Quality control

Analyse instrument and reagent blank and reference samples of comparable matrix having reliably known contents of the element species to be determined in parallel with all the series of samples for analytical quality control. Subject the reagent blank and reference samples to all the steps in the method.

Check regularly within suitable short intervals (e.g. after 5 samples), a blank sample. Memory effects in the sample delivery system may influence the results of samples analysed after measurement of samples with high mass fractions of MMHg. Prolonged washout times may have to be applied. Test the system for wash out times using a sample with high MMHg mass fraction.

If the MMHg mass fractions obtained using the two isotope ratios differ more than 10 %, apply corrective measures. Check that the sample has been spiked with the appropriate amount to achieve approximate abundance matching of the isotopes in the sample. In addition, check the integration and other potential sources of error.

Since there is no sample cleanup (i.e. removal of fat) pay attention to the condition of the GC-liner when running multiple samples with high fat content.

7 Calculation

Using the peak areas found in the analyses, calculate the mass fraction of MMHg as Hg in the sample, w_s in mg/kg with the isotope dilution in Formula (2) [6]. Other versions of the same formula may also be used for calculation [7] [8].

$$w_S = w_{Sp}\frac{m_{Sp}}{m_S}\frac{M_S}{M_{Sp}}\frac{A_{Sp}^{b}}{A_S^{a}}\left(\frac{R_m - R_{Sp}}{1 - R_m \cdot R_S}\right)\frac{1}{1000} \quad (2)$$

where

w_{Sp} is the MMHg mass fraction in the spike solution, in ng/g (µg/kg);

m_S is the mass of test portion, in g;

m_{Sp} is the mass of added spike solution, in g;

M_S is the molar mass of MMHg in sample, in g/mol;

M_{Sp} is the molar mass of MMHg in spike solution, in g/mol;

A_S^a is the percent of reference isotope a (^{200}Hg or ^{202}Hg) in sample;

A_{Sp}^b is the percent of reference isotope b (^{201}Hg) in spike solution;

R_S is the isotope ratio of Hg-isotope *a* and *b* in sample, see Formula (3);

R_{Sp} is the isotope ratio of Hg-isotope *a* and *b* in spike solution, see Formula (4);

R_m is the measured isotope ratio between Hg-isotope *a* and *b* in spiked sample, see

Formula (5);

1/1000 is the calculation factor from µg/kg to mg/kg.

$$R_S = \frac{A_S^b}{A_S^a} \quad (3)$$

where

A_S^b is the percent of reference isotope b (^{201}Hg) in sample;

A_S^a is the percent of reference isotope a (^{200}Hg or ^{202}Hg) in sample.

$$R_{Sp} = \frac{A_{Sp}^a}{A_{Sp}^b} \quad (4)$$

where

A_{Sp}^a is the percent of reference isotope a (^{200}Hg or ^{202}Hg) in spike solution;

A_{Sp}^b is the percent of reference isotope b (^{201}Hg) in spike solution.

$$R_m = \frac{N_m^a}{N_m^b} \quad (5)$$

where

N_m^a is the peak area of the Hg-isotope a (^{200}Hg or ^{202}Hg) in the spiked sample;

N_m^b is the peak area of the Hg-isotope b (^{201}Hg) in the spiked sample.

Use both the ^{202}Hg/^{201}Hg and ^{200}Hg/^{201}Hg ratios for calculation and report the mean value of MMHg from these calculations. If the MMHg mass fractions obtained using the two isotope ratios differ more than 10 %, apply corrective measures (6.6.3). A higher tolerance for the difference can be acceptable in respect to the variance of the results for the matrix.

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

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 [5].

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 4 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 4 in not more than 5 % of the cases.

Table 4 — Mean values, repeatability (*r*) and reproducibility (*R*) limits

Sample (all lyophilized)	$\overline{x}$ mg/kg	*r* mg/kg	*R* mg/kg
Mussel tissue	0,035	0,003	0,014
Squid muscle	0,185	0,008	0,011
Crab claw muscle	0,307	0,012	0,032
Dog fish liver[a]	1,329	0,061	0,104
Whale meat	1,454	0,074	0,140
Cod muscle	1,976	0,064	0,142
Greenland Halibut muscle	3,575	0,076	0,244
[a] The dog fish liver sample was NRCC DOLT 4 with a certified value of 1,33 mg/kg			

9 Test report

The test report should fulfil the requirements in EN ISO/IEC 17025 [9] 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 results obtained and the units in which they are specified;

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

e) the date when the analysis was finished;

f) 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)

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 according to ISO 5725-2 [1] among eight laboratories using freeze-dried samples, performed in 2013 under a mandate given by the European Commission. The results are given in Table A.1 and A.2, see also [5].

Table A.1 — Precision data

Parameter	Mussel tissue	Squid muscle	Crab claw meat	Whale meat	Cod muscle	Greenland halibut muscle
Number of reporting laboratories	8	8	8	8	8	8
Number of laboratories after elimination of outliers	7[a]	8	8	8	8	8
Number of outlying laboratories	1	0	0	0	0	0
Number of replicates after outlier rejection	14	16	16	16	16	15
Numbers of rejected replicates	2[a]	0	0	0	0	1
Mean value $\bar{x}$, mg/kg	0,035	0,185	0,307	1,454	1,976	3,575
Repeatability limit *r,* mg/kg	0,003	0,008	0,012	0,074	0,064	0,076
Repeatability standard deviation s(*r*), mg/kg	0,003	0,008	0,012	0,074	0,064	0,076
RSD(*r*), %	8,67	4,53	3,97	5,11	3,24	2,14
Reproducibility limit *R,* mg/kg	0,014	0,011	0,032	0,140	0,142	0,244
Reproducibility standard deviation s(*R*), mg/kg	0,014	0,011	0,032	0,14	0,142	0,244
RSD(*R*), %	41,73	5,75	10,54	9,66	7,17	6,9
Horwitz value according to Horwitz [10]	26,53	20,62	19,11	15,12	14,44	13,21
HorRat value according to Horwitz [10]	1,57	0,28	0,55	0,64	0,50	0,52
Horwitz value according to Thompson [11]	22,00	20,62	19,11	15,12	14,44	13,21
HorRat value according to Thompson [11]	1,90	0,28	0,55	0,64	0,50	0,52

[a] MMHg mass fraction in the sample was not quantifiable by one of the participants.

Table A.2 — Trueness based on NRCC DOLT 4 (Dog fish liver)

Parameter	MMHg
Found mean value (mg/kg)	1,329
Certified value (mg/kg)	1,33
S_R (mg/kg)	0,104
Z-score	- 0,01

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