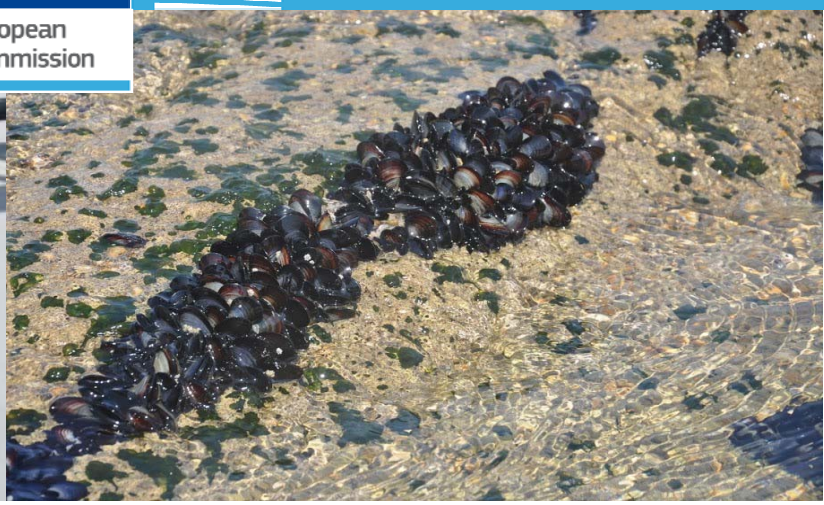

JRC TECHNICAL REPORTS

Determination of methylmercury in seafood by direct mercury analysis: Standard operating procedure

Detailed information on the organisation and outcome of the collaborative trial, IMEP-115, organised to validated the present standard operating procedure can be found in the report EUR 25830 EN 2013

J. Calderón, S. Gonçalves,
F. Cordeiro, B. de la Calle

February 2013

Report JRC 80259 EN

Joint Research Centre

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information
Dr. Fernando Cordeiro
Joint Research Centre, Retieseweg 111, 2440 Geel, Belgium
E-mail: maria.de-la-calle@ec.europa.eu
Tel.: +32 (0) 14 571687
Fax: +32 (0) 14 571865

http://irmm.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

JRC

Printed in Belgium

STANDARD OPERATING PROCEDURE

DETERMINATION OF METHYLMERCURY IN SEAFOOD BY ELEMENTAL MERCURY ANALIZER

J. CALDERÓN [A]

S. GONÇALVES [B]

F. CORDEIRO [C]

B. DE LA CALLE [C]

[A] LABORATORI AGÈNCIA SALUT PÚBLICA DE BARCELONA, SPAIN

[B] INSTITUTO PORTUGUES DO MAR E DA ATMOSFERA, PORTUGAL

[C] EUROPEAN COMMISSION, DIRECTORATE GENERAL JOINT RESEARCH CENTRE, INSTITUTE FOR REFERENCE MATERIALS AND MEASUREMENTS, BELGIUM

CONTENTS

1 EXECUTIVE SUMMARY

Under request by Directorate General for Health and Consumers (DG SANCO) a collaborative trial, IMEP-115, was conducted in accordance with international protocols by the European Union Reference Laboratory for Heavy Metals in Feed and Food (EURL-HM), to determine the performance characteristics of an analytical method for the determination of methylmercury in seafood.

IMEP-115 was organised in support to the Commission Regulations 1881/2006 and 882/2004. The method is based on a double liquid-liquid extraction, first with an organic solvent and then with a cysteine solution. The final quantification is done with a direct mercury analyzer.

The repeatability relative standard deviation (RSD_r) ranged from 3.9 to 12.3 % while the reproducibility relative standard deviation (RSD_R) ranged from 8.4 to 24.8 %.

The method demonstrates to have acceptable precision for all test materials, thus it fit for its intended analytical purpose.

Detailed information on the organisation and outcome of the collaborative trial, IMEP-115, can be found in the report EUR 25830 EN 2013 [1].

2 INTRODUCTION

Methylmercury, sometimes written as MeHg, is a shorthand for "monomethylmercury", and would be even more correctly "monomethylmercuric cation". As a positively charged ion it readily combines with anions and has very high affinity for sulfur-containing anions, particularly the thiol (-SH) groups on the amino acid cysteine and hence in proteins containing cysteine, forming a covalent bond.

This standard operating procedure (SOP) describes the analysis of methylmercury based on a double liquid-liquid extraction, firstly with organic solvent and subsequently with a cysteine solution. The instrumental analysis is performed using an elemental mercury analyser [2].

Elemental mercury analyser, also known as automated or direct mercury analyser, is a single purpose atomic absorption spectrophotometer for mercury determination. It is designed for the direct mercury determination in solid and liquid samples without a need of sample chemical pre-treatment.

This analyser is based on a sample drying and subsequent thermal decomposition, followed by an 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 released by temperature and detected by atomic absorption at 253.7 nm.

The analytical method would extract other organic mercury species in case may be present, but it can be all considered as methylmercury because it represents almost the totally of the organic mercury in food samples.

3 APPLICATION SCOPE

The application scope of this SOP is the determination of methylmercury in seafood/fishery products.

Instrumental concentration range is 1 μg/L – 100* μg/L as Hg

Quantification limit is 0,010 mg/kg of methylmercury (as Hg)

* Samples with Hg concentrations above this level would be diluted (or reanalyse with less volume) appropriately to ensure measurement within the calibration range.

4 MATERIAL AND EQUIPMENTS

Automated mercury analyser AMA-254 or DMA-80 or equivalent

Own equipment consumables (cuvettes for 0.5 mL)

Analytical balance with at least 1 mg of resolution

Centrifuge (minimum 4000 rpm)

Micropipette covering at least the range of 50-1000 µL, with 1 µL of resolution.

Micropipette covering at least the range of 5 – 10 mL, with minimum resolution of 0,05 mL.

Note: Glass volumetric pipettes may be used instead of micropettes.

Material commonly used in laboratory. All volumetric glassware is a Class A, and has to be previously acid washed (24 hours immersed in 10 % HNO_3).

5 REAGENTS AND STANDARDS

Purified water type I (ASTM).

Mercury Standard of 1000 mg/L, quality AA

Methylmercury chloride purity > 95 %

Nitric acid nitric 65%, suprapur

Hydrochloric acid (min. 32 %)

Potassium dichromate

Hydrobromic acid (47 %)

Toluene p.a.

L-Cysteine monohydrate hydrochloride

Sodium Sulphate anhydrous

Sodium acetate anhydrous

Oxygen: quality N-50 .

Note: reagents used should have analytical reagent qualities or equivalent/superior

5.1 Reagents preparation

5.1.1 Solution of $K_2 Cr_2 O_7$ 1% ($^w/_v$).

Weigh 1.0 g of potassium dichromate in a 100 mL volumetric flask, add 50 mL of water purified type I, stir it well until completely dissolved and make up to volume with the purified water. Store in the fridge (2-10 ºC) up to a maximum of two years.

5.1.2 Hydrochloric acid diluted 1:1.

Mix equal volumes of concentrated hydrochloric acid and purified water.

5.1.3 L-cysteine solution at 1 % ($^w/_v$):

In a 100 mL beaker weigh 1.0 g of L-Cysteine monohydrate chlorhydrate, 12,5 g of the sodium sulphate and 0,8 g the sodium acetate. Add about 75 mL of purified water. Stir until complete dissolution. Transfer this solution completely to a 100 mL volumetric flask, and make up to volume with the purified water. This solution can be stored for 1 day at ambient temperature. Other preparation volumes may be used as long as they keep the proportions.

5.2 Intermediate standard solutions

5.2.1 Standard solution of 10 mg Hg /L.

Into a 100 mL volumetric flask, add 1.00 mL of the commercial solution of Hg 1000 mg/L, add 1 mL of $K_2 Cr_2 O_4$ 1% ($^w/_v$) solution, about 2 mL of hydrochloric acid 1:1, and make up to volume with the purified water. This solution is stable in a glass container in the fridge (2-10 ºC) for 6 months. Potassium dichromate is not need to be added if this standard solution is prepared the same day of instrumental calibration is performed.

5.2.2 Standard solution of 500 µg Hg /L.

Introduce 2,50 mL of 10 mg/L mercury standard solution into a 50 mL volumetric flask, add 1 mL of hydrochloric acid 1:1, and make up to volume with the purified water. This solution is stable in a glass container in the fridge (2-10 ºC) for 2 months.

5.3 Calibration Standard Solutions

NOTE: All calibration standard solutions are newly prepared for each calibration. Other volumes of preparation are suitable provided that they maintain the proportions above described.

5.3.1 Calibration standard of 100 µg Hg /L.

Introduce 10 mL of 500 µg/L mercury standard solution into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % ($^{w}/_{v}$) (4.1.3).

5.3.2 Calibration standard of 75 µg Hg /L.

Introduce 7.5 mL of 500 µg/L mercury standard solution into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.3 Calibration standard of 50 µg Hg /L (*equivalent to QC2 solution in 4.4.1*)

Introduce 5.0 mL of 500 µg/L mercury standard solution into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.4 Calibration standard of 25 µg Hg /L

Introduce 2.5 mL of 500 µg/L mercury standard solution into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.5 Calibration standard of 15 µg Hg /L

Introduce 1.5 mL of 500 µg/L mercury standard solution into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.6 Calibration standard of 10 µg Hg /L

Introduce 1.0 mL of 500 µg/L mercury standard solution into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.7 Calibration standard of 5 µg Hg /L

Introduce 0.5 mL of 500 µg/L mercury standard solution into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.8 Calibration standard of 2.5 µg Hg /L

Introduce 2.5 mL of 50 µg/L calibration standard into a 50 mL volumetric flask and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.9 Calibration standard of 1.0 µg Hg /L (*equivalent to QC1 solution in 4.4.2*)

Introduce 1.0 mL of 50 µg/L calibration standard into a 50 mL volumetric flask and make up to volume with L-cysteine solution 1 % (4.1.3).

5.3.10 Blank (for calibration)

The L-cysteine solution 1 % ($^{w}/_{v}$) is used as blank (level 0) for instrumental calibration.

5.3.11 External standard solution

Each calibration curve need to be compared against an external solution to demonstrate absence of error in intermediate standard solutions preparation. Several options are possible, for example:

Prepare a 50 µg/L external standard of Hg as described in 4.3.3, but using intermediate solutions prepared from a different brand or different batch of the commercial 1000 mg/L standard than that used for calibration standards. Another option is that the 50 µg/L external standard of Hg can be prepared from a standard solution of MeHg. In that case, introduce 2.7 mL of 4.5.2 solution into a 50 mL volumetric flask and make up to volume with L-cysteine solution 1 % (4.1.3).

The analysis of a certified reference material could also be an option.

5.4 Internal quality control solutions

Due to the response of elemental mercury analysers is highly stable, there is no need to recalibrate for each analytical sequence. It is for this reason that some control solutions are used to ensure the validity of that former calibration. QC1 solution ensures that the quantification at low level is still correct. QC2 demonstrates that response is stable at higher concentrations too and at the same time, because it is placed at the end of the sequence, that there is not any drift uncontrolled.

5.4.1 Intermediate level solution QC2 (50 µg Hg /L)

Introduce 5.0 mL of the 500 µg/L mercury standard solution (see section 4.2.2) into a 50 mL volumetric flask, and make up to volume with L-cysteine solution 1 % (4.1.3). This solution is newly prepared for each analytical sequence.

5.4.2 Quantification limit solution QC1 (1 µg Hg /L)

Introduce 1.0 mL of QC2 solution into a 50 mL volumetric flask and make up to volume with L-cysteine solution 1 % (4.1.3). This solution is newly prepared for each analytical sequence.

5.5 Spiking solutions

The spike will be done with a standard solution of methylmercury.

5.5.1 Standard solution of 80 mg $_{MeHg}$ /L (*equivalent to 74,4 mg/L expressed as Hg*).

Into a 250 mL volumetric flask, accurately weight 0,024 g of methylmercury chloride (consider its purity for the final concentration), add about 4 mL of hydrochloric acid 1:1 and about 200 mL of purified water. Shake thoroughly until complete dissolution and make up to volume with purified water. This solution is stable in a glass container in the fridge (2-10 ºC) for 1 year.

5.5.2 Spiking solution of 1 mg $_{MeHg}$ /L (*equivalent to 0,93 mg/L expressed as Hg*).

Introduce 625 µL of 80 mg/L methylmercury standard solution (see section 4.5.1) into a 50 mL volumetric flask, add about 1 mL of hydrochloric acid 1:1 and make up to volume with purified water. This solution is stable in a glass container in the fridge (2-10 ºC) for 3 months.

6 OPERATIONAL PROCEDURE

Switch on the instrument and let it reach the working temperatures. “Clean” the system (some software have a “clean” option which means that the temperature of catalyser and amalgamator is raised for a determined time). Subsequently introduce about 0,5 mL of water into the cuvette and press analysis button. Finally, analyse a Reagent Blank (with the empty cuvette, press analysis button). The result should be ≤ 0,3 µg/L, if not, clean again the system as described before. It should not take more than 2 - 3 minutes between this step and the one described in section 5.4 (or 5.3 when calibrating).

6.1 Instrumental characteristics

The following characteristics are only a guide, and they may change between instrument brands.

Drying time: 250 seconds

Drying temperature: 285 ± 25 ºC

Decomposition time: 150 seconds

Decomposition temperature: 725 ± 25 ºC.

Volume of analysis (for samples, standards and blanks): 500 µL

Cell to be used: Because the calibration range is quite large (due to the wide variability of concentrations to consider in seafood samples), is not easy to obtain always a good adjustment of the calibration curve for all levels, especially at low levels. If this is the case, to improve such adjustment, two different calibration curves are needed, and this implies to work with the first cell at low levels and with the second cell for higher concentrations.

Standards used for calibration range with first cell: 1, 2.5, 5, 10, 15, 25 µg/L (plus solution 4.1.3 as the blank). Samples with concentration above 25 µg/L in the extract, will be quantified with the calibration using the second cell.

Standards used for calibration range with second cell: 15, 25, 50, 75 and100 µg/L (a range of overlap between the two calibration lines 15 – 25 µg/L is recommended because improves the comparison between the two calibration in the exchange zone.)

6.2 Sample Preparation

Into a 50 mL centrifuge tube, weight 0,7 – 0,8 g of sample (or 0,2 g in the case of lyophilised samples, plus 0.5 mL of purified water). Add 10 mL of hydrobromic acid and shake it manually. Add 20 mL of toluene, shake vigorously (i.e. vortex) for at least 2 minutes. Centrifuge for 10 minutes at 3000 rpm. Take about 15 mL from the above organic phase and place it into a 50 mL containing 6.0 mL of L-cysteine solution 1 % (4.3.1).

Add about 15 mL more of toluene into the initial centrifuge tube (containing still the hydrobromic acid phase), and repeat a second extraction with the organic phase. After centrifugation, take the remaining upper organic phase and place it into the previous 50 mL tube with cysteine solution. Shake it vigorously (i.e. vortex) for at least 2 minutes and centrifuge for 10 minutes at 3000 rpm. With a Pasteur glass pipette, take an aliquot of 2-3 mL from the lower phase with cysteine (which contains already the extracted organic mercury) and place it into a glass vial with cap (i.e. vial for chromatography). This extract is stable for one week in the fridge.

In case an emulsion is formed at the interface between the organic toluene phase and the hydrobromic acid phase, tap a few times the container against the table, centrifuge again at a higher speed (about 5000 rpm for 10 min.). After a few moments the separation should be completely clear.

Parallel to the extraction of the samples, a blank is analysed too (sample extraction without sample). In addition, at least one of the samples will be weight again and before adding the reagents, spike it with the spiking solution described in section 4.5.2. As a guide, if spiked sample is a depredator (tuna, shark, etc.) higher levels of mercury (and methylmercury) are expected, so a volume of addition between 400 - 600 µL of the above spiking solution could be a feasible addition. For smaller species, 100 – 300 µL can be a good option.

Instead of a spiked sample, a reference material with a known content of methylmercury, is also a good option for trueness evaluation within the internal control, although working with additions allows more variability in internal control in both matrix and concentration level tested. Whenever possible, the CRM should match the test samples.

6.3 Calibration

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 maintain for that period, provided quality controls for each sequence are satisfactory. Nevertheless, if gold amalgamator is changed, response may change and a new calibration will be needed.

To do it, analyse 500 µL of each calibration solution (from blank to 100 µg/L). Refer to section 5.1 to select the corresponding standards for each calibration curve. See section 7.2 to accept the calibration curve.

6.4 Analytical sequence

Use the following sequence as a guide:

Quantification level solution QC1 (1 µg/L) (see section 4.4.1).

Blank of method

Samples (and among them, one spiked sample as described in section 5.2).

Intermediate level solution QC2 (50 µg/L) (see section 4.4.2)

6.5 Information to register

All data to ensure traceability of results must be registered, i.e., date, analyst, used instruments (scale, pipettes,etc.), corresponding calibration curve, evaluation of quality controls, etc., as well as any other needed comment.

7 RESULTS

7.1 Calculation

Results are directly obtained in µg/L of Hg in the extract. Calculation is as follows:

$$\text{Me Hg (mg/kg as Hg)} = \frac{C \times 6 \times D}{w \times 1000}$$

On:

C: Concentration into the extract expressed in µg/L of Hg.

D: dilution factor, if needed

6: Volume of L-cysteine solution 1 %.

w: weight of sample in grams

7.2 Expression

Results of methylmercury (as Hg) are expressed in mg/kg ± uncertainty of the measurement. The number of decimal figures is 3 below 0,25 mg/kg and 2 decimal figures from 0,25 mg/kg.

Results below quantification limit will be informed as < 0,010 mg/kg.

8 INTERNAL CONTROL FOR QUALITY EVALUATION

To accept the results, it is needed meet the following criteria:

8.1 Instrument verification:

Instrument verification is done with control solutions at the beginning and the end of the analytical sequence. Solution QC1 will ensure that sensitivity and so signal stability has been maintained since last calibration. Solution QC2 at the end of the sequence will ensure that the behaviour of the instrument is stable along all the sequence.

Acceptance criteria QC 1 (1,0 µg/L): 0,85 – 1,15 µg/L

Acceptance criteria QC 2 (50 µg/L): 45 – 55 µg/L

8.2 Calibration curve:

Calculate relative residual error (calculated concentration compared to the theoretical value) for each calibration level.

Acceptance criteria:

For STD 1 µg/L: % residual error < 15 %

For all other standards: % residual error ≤ 10 %

External standard (see section 4.3.11) to accept calibration: expected value ± 10 %

8.3 Absence of contamination

Blank of method extract should be not higher that 1/3 of quantification limit.

8.4 Trueness/Precision:

Calculate % of recovery from the spiked sample (or in the case of having used a reference material, compared the obtained value with the certified one).

$$\% \text{ recovery} = \frac{\text{Cadd} - \text{Csample}}{(\text{Ctheor})} x100$$

where:

Cadd: concentration of methylmercury (as Hg) obtained in the spiked sample (or reference material)

Csample: concentration of methylmercury (as Hg) in the original sample without spiking (do not consider in the case of having used a reference material)

Ctheor: Theoretical concentration expected Ctheor=v*C/p (considering volume (v) in mL, concentration of spiking standard (C) in mg/L expressed as Hg, and weight of sample (w) in grams).

Acceptance criteria: As a general range, 85 – 115 % of recovery should be obtained.

If the method has been validated by the laboratory, the percentage of recovery obtained could be compared against the mean recovery obtained in validation ± 2s (where standard deviation is obtained from validation too. So in that way, trueness and precision are evaluated at the same time.

NOTE: All method performance characteristics related to precision will be estimated from the results of collaborative trial.

9 BIBLIOGRAPHY

[1] "IMEP-115: Determination of methylmercury in seafood - A collaborative trial report", F. Cordeiro *et al*., EUR 25830 EN 2013.

[2] "Total an organic mercury, selenium and alfa-tocopherol in some deep water fish species", C. Alfonso *et al*. Journal of the Science of Food and Agriculture. 88:2543-2550 (2008)

European Commission
JRC – Joint Research Centre – Institute for Reference Material and Measurements
Title: Determination of methylmercury in seafood by direct mercury analysis: Standard operating procedure

Author(s): J. Calderón, S. Gonçalves, F. Cordeiro, B. De la Calle

2013 – 12 pp. – 21.0 x 29.7 cm

JRC –Technical Research series

Abstract

This standard operating procedure (SOP) describes the analysis of methylmercury based on a double liquid-liquid extraction, firstly with organic solvent and subsequently with a cysteine solution. The instrumental analysis is performed using an elemental mercury analyser. The present SOP was followed during a collaborative trial (IMEP-115) by all participants, having in mind the establishment of all method performance characteristics, related to precision. The outcome was that, while respecting the presented extraction procedure, the method has adequate precision and trueness, hence it fits its intended analytical purpose.

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new standards, methods and tools, and sharing and transferring its know-how to the Member States and international community.

Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security including nuclear; all supported through a cross-cutting and multi-disciplinary approach.