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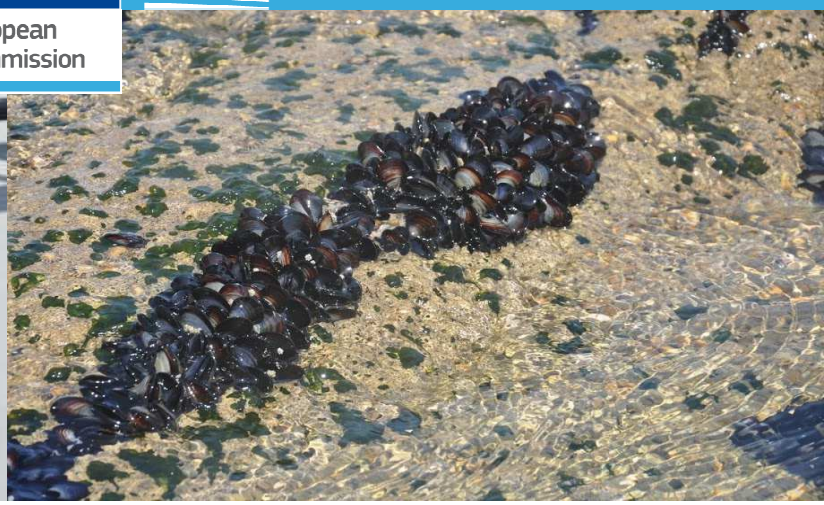

J R C T E C H N I C A L R E P O R T S

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

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

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STANDARD OPERATIONAL PROCEDURE

DETERMINATION OF METHYLMERCURY IN SEAFOOD BY ELEMENTAL MERCURY ANALIZER

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(This report is an annex to the collaborative trial report "IMEP-115: Determination of methylmercury in seafood" EUR 25830 EN, February 2013)

CONTENTS

1 EXECUTIVE SUMMARY

This standard operational 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 the collaborative trial (IMEP-115: Determination of methylmercury in seafood, [1]) 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.

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 operational 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^{-1} – 100* μg L^{-1} as Hg

Quantification limit is 0.010 mg kg^{-1} of methylmercury (as Hg)

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

4 MATERIAL AND EQUIPMENTS

Automated mercury analyser (AMA-254, 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

The concentration of mercury in the reagents and water used shall be low enough not to affect the results of the determination. All reagents shall be of analytical grade or similar unless otherwise specified.

Use water conforming to grade 2 of EN ISO 3696.

Mercury standard 1000 mg L^{-1} of Hg (quality AA)

Methylmercury chloride (purity > 95 %)

Nitric acid nitric 65 % (suprapur quality)

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)

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 hydrochloride, 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 L^{-1} of Hg

Add 1.00 mL of the commercial solution 1000 mg L^{-1} of Hg in a 100 mL volumetric flask, 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 purified water. This solution is stable in a glass container in the fridge (2-10 ºC) for 6 months. There is no need to add $K_2 Cr_2 O_4$ if this standard solution is prepared the day that the instrumental calibration is performed.

5.2.2 Standard solution of 500 µg L^{-1} of Hg

Place 2.50 mL of 10 mg L^{-1} mercury standard solution (5.2.1) in a 50 mL volumetric flask add 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 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 described above.

5.3.1 Calibration standard of 100 µg L^{-1} of Hg

Place 10 mL of 500 µg L^{-1} mercury standard solution (5.2.2) into a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.2 Calibration standard of 75 µg L^{-1} of Hg

Place 7.5 mL of 500 µg L^{-1} mercury standard solution (5.2.2) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.3 Calibration standard of 50 µg L^{-1} of Hg (*equivalent to QC2 solution in 5.4.1*)

Place 5.0 mL of 500 µg L^{-1} mercury standard solution (5.2.2) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.4 Calibration standard of 25 µg L^{-1} of Hg

Place 2.5 mL of 500 µg L^{-1} mercury standard solution (5.2.2) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.5 Calibration standard of 15 µg L^{-1} of Hg

Place 1.5 mL of 500 µg L^{-1} mercury standard solution (5.2.2) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.6 Calibration standard of 10 µg L^{-1} of Hg

Place 1.0 mL of 500 µg L^{-1} mercury standard solution (5.2.2) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.7 Calibration standard of 5 µg L^{-1} of Hg

Place 0.5 mL of 500 µg L^{-1} mercury standard solution (5.2.2) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.8 Calibration standard of 2.5 µg L^{-1} of Hg

Place 2.5 mL of 50 µg L^{-1} mercury standard solution (5.3.3) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.9 Calibration standard of 1.0 µg L^{-1} of Hg (*equivalent to QC1 solution in 5.4.2*)

Place 1.0 mL of 50 µg L^{-1} mercury standard solution (5.3.3) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3).

5.3.10 Blank (for calibration)

The L-cysteine 1 % (w/v) solution (5.1.3) 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 systematic error in intermediate standard solutions preparation. Several options are possible, for example:

Prepare a 50 µg L^{-1} of Hg external calibration standard solution (5.3.3) but using intermediate solutions prepared from a different brand or different batch of the commercial 1000 mg L^{-1} of Hg standard than that used for calibration standards. Another option is that the 50 µg L^{-1} external standard of Hg can be prepared from a standard solution of methylmercury (MeHg). In that case, place 2.7 mL of 5.5.2 solution in a 50 mL volumetric flask and make up to volume with the L-cysteine 1 % (w/v) solution (5.1.3).

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

5.4 Internal quality control solutions

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 that former calibration. QC1 solution ensures that the quantification at low level is still correct. QC2 demonstrates that response is stable at higher concentrations and, at the same time, because it is placed at the end of the sequence, that there is not any uncontrolled drift.

5.4.1 Intermediate level solution QC2 (50 µg L^{-1} of Hg)

Place 5.0 mL of the 500 µg L^{-1} mercury standard solution (5.2.2) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3). This solution is newly prepared for each analytical sequence.

5.4.2 Quantification limit solution QC1 (1 µg L^{-1} of Hg)

Place 1.0 mL of QC2 solution (5.4.1) in a 50 mL volumetric flask and make up to volume with L-cysteine 1 % (w/v) solution (5.1.3). This solution is newly prepared for each analytical sequence.

5.5 Spiking solutions

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

5.5.1 Standard solution of 80 mg L^{-1} of MeHg (*equivalent to 74.4 mg L^{-1} expressed as Hg*).

Weigh accurately 0.024 g of methylmercury chloride (consider its purity for the final concentration) in a 250 mL volumetric flask, add about 4 mL of hydrochloric acid 1:1 and 200 mL of purified water. Shake thoroughly until complete solubilisation 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 L^{-1} of MeHg (*equivalent to 0.93 mg L^{-1} expressed as Hg*).

Place 625 µL of 80 mg L^{-1} methylmercury standard solution (5.5.1) in a 50 mL volumetric flask add 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, place 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^{-1} of Hg, 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 6.4 (or 6.3 when calibrating).

6.1 Instrumental characteristics

The following characteristics are only a guide, and they may change depending on the 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 achieve a good adjustment of the calibration curve for all concentration levels, especially at low concentration levels. In this case, to improve such adjustment, two different calibration curves are needed, and this implies the use of two different cells for low and high concentrations, respectively.

Standards used for calibration range with the first cell (low concentration level): 1, 2.5, 5, 10, 15 and 25 µg L^{-1} of Hg (plus solution 5.1.3 as the blank). Samples with concentration above 25 µg L^{-1} in the extract will be quantified with the calibration curve constructed with the second cell.

Standards used for calibration range with the second cell (high concentration level): 15, 25, 50, 75 and 100 µg L^{-1} (a range of overlap between the two calibration lines 15 – 25 µg L^{-1} is recommended because improves the comparison between the two calibration curves in the exchange zone.)

6.2 Sample Preparation

Weigh 0.7 – 0.8 g of sample (or 0.2 g in the case of lyophilised samples, plus 0.5 mL of purified water) in a 50 mL centrifuge tube, add 10 mL of hydrobromic acid and shake it manually. Add 20 mL of toluene and shake it 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 centrifuge tube containing already 6.0 mL of L-cysteine 1 % (w/v) solution (5.1.3).

Add 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 centrifuge tube with the L-cysteine 1 % (w/v) solution (5.1.3). 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 the L-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 two phases should be completely separated.

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 of 1 mg L^{-1} of MeHg (5.5.2). 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 (5.5.2) could be a feasible addition. For smaller species, 100 – 300 µL can be a good option.

A (certified) reference material (CRM) 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 the gold amalgamator is changed, response may change and a new calibration will be needed.

In such a case, analyse 500 µL of each calibration solution (from blank to 100 µg L^{-1} of Hg). Refer to section 6.1 to select the corresponding standards for each calibration curve. See section 8.2 to accept the calibration curve.

6.4 Analytical sequence

Use the following sequence as a guide:

Quantification level solution QC1 (1 µg L^{-1} of Hg, 5.4.2).

Blank of method

Samples (and among them, one spiked sample as described in section 6.2).

Intermediate level solution QC2 (50 µg L^{-1} of Hg, 5.4.1)

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^{-1} of Hg in the extract. Calculation is as follows:

$$\text{MeHg (mg kg}^{-1}\text{ as Hg)} = \frac{C \times 6 \times D}{w \times 1000}$$

Where:

C: Concentration into the extract expressed in µg L^{-1} of Hg,

D: dilution factor, if needed,

6: Volume of L-cysteine 1 % (w/v) solution (5.1.3),

w: weight of sample in grams.

7.2 Expression

Results of methylmercury (as Hg) are expressed in mg kg^{-1} ± uncertainty of the measurement. The number of decimal figures is 3 below 0.25 mg kg^{-1} and 2 decimal figures from 0.25 mg kg^{-1} onwards.

Results of MeHg (as Hg) below quantification limit will be reported as < 0.010 mg kg^{-1}.

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 internal 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 the 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^{-1} of Hg, 5.4.2): 0.85 – 1.15 µg L^{-1}

Acceptance criteria QC 2 (50 µg L^{-1} of Hg, 5.4.1): 45 – 55 µg L^{-1}

8.2 Calibration curve:

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

Acceptance criteria:

For QC1 (1 µg L^{-1} of Hg): % residual error < 15 %

For all other standards: % residual error ≤ 10 %

External standard solution (5.3.11) to accept calibration: expected value ± 10 %

8.3 Absence of contamination

The concentration of methylmercury in the blank should be ≤ 1/3 of the quantification limit.

8.4 Trueness/Precision:

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

$$\% \text{ recovery} = \frac{C_{add} - C_{sample}}{(C_{theor})} x100$$

Where:

C_{add}: concentration of methylmercury (as Hg) obtained in the spiked sample (or for the certified reference material)

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

C_{theor}: Theoretical concentration expected C_{theor}=v*C/w (considering volume (v) in mL, concentration of spiking standard (C) in mg kg^{-1} expressed as Hg, and weight of sample (w) in grams).

Acceptance criteria: recovery should fall in the range 85 – 115 %.

All method performance characteristics related to precision were estimated from the results of a collaborative trial organised by the Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a General Directorate of the European Commission, in 2013 with participating laboratories from Belgium, Czech Republic, France, Italy, Poland, Portugal and Spain.

The statistical analysis of the data was done in accordance with ISO 5725-2 [3]. Further details regarding the outcome of the collaborative trial can be found in the final report [1].

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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.

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