UMWELTPROBENBANK DES BUNDES

Guidelines for Chemical Analysis

Quantification of Methyl Mercury Compounds in Environmental Samples by ICP-MS

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Guidelines for sampling, transport, storage and chemical characterisation of environmental and human samples
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1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for the monitoring of the environment. It is in the responsibility of the Federal Ministry for the Environment, Nature Protection and Reactor Safety (BMU) and technically and administratively coordinated by the Federal Environment Agency (Umweltbundesamt). The ESB collects ecologically representative environmental specimens as well as human samples, stores them and examines the archived material for environmental relevant substances.

The long-term storage is performed under conditions that exclude a change of state or a loss of chemical characteristics as far as possible during a period of several decades. By this means the archive provides specimens for a retrospective monitoring of such substances, whose hazard potential for the environment or human health are not yet known.

Comprehensive information on the German ESB is available at www.umweltprobenbank.de (English language pages available).

2 General information

This guideline quantifying the content of methyl mercury compounds in environmental samples by means of mass spectrometry following ionisation in inductively coupled plasma (ICP-MS: inductively coupled plasma mass spectrometry), is a continuation of the methodological guidelines developed by the Federal Environmental Specimen Bank for analysing environmental samples (UMWELTBUNDESAMT 1996). The procedure described there is the cold-vapour atomic-absorption spectrometry method (CV-AAS). However, in view of its higher specificity and sensitivity, analysis by means of ICP-MS is now preferred for the Environmental Specimen Bank demands.

The present guideline has been optimised for use with the following types of sample: zebra mussels, common mussels and roe-deer liver. The material is used as deep-frozen pulverised homogenate. In principle, the method described here can also be used for other types of biological samples. Where samples are used for which no empirical data are available, a suitable process for validation should also be carried out (see section 6).

The lower range of application of the process described depends on the matrix in question and the types of interference connected therewith. The limit of detection for the standard addition process is approximately 1 ng/g with reference to the dry mass.

3 Description of method

In order to determine the content of methyl mercury compounds in environmental samples, it is necessary to separate the organic mercury species from the inorganic, since both of these may be present in samples. The mercury species are separated by means of an ion exchanger following hydrochloric acid extraction of the sample material. Under the selected conditions, the inorganic species of mercury are bound whereas the organic are not retained and pass through the ion exchanger. The eluate is collected and subjected to UV digestion in order to destroy interfering matrix components of the sample by means of oxidation. The eluate thus treated then is analysed by ICP-MS using the standard addition method in order to determine its mercury content. From the mercury data the concentration of the methyl mercury cation is calculated and reported.

The extraction and the ion exchange are carried out in the absence of light in order to prevent the degradation of methyl mercury compounds by photochemical processes. The whole procedure is an operationally defined method because it is based on the assumption that the entire mercury fraction isolated from the sample under these conditions consists of methyl mercury compounds. Theoretically, other organic mercury compounds could also be present. However, existing scientific literature does not indicate that other organic mercury species are present in significant quantities (SCERBO ET AL. 2005).

4 Apparatus and reagents

4.1 Vessels for element solutions

The stability of diluted mercury solutions (both sample and standard solutions) is determined substantially by the material of the vessels used. The suitability of the material for the intended purpose must always be ensured beforehand. For determining elements in the trace range, vessels of glass or polyvinyl chloride (PVC) should not be used. Vessels made of perfluoralkoxy plastics (PFA), hexafluoro-ethylene-propylene (FEP) or quartz are more suitable. In many cases, high-density polyethylene (PE, e.g. HDPE vessels which are used for scintillation measurements) and polypropylene (PP) may also be used. When they are being re-used, the vessels must be rinsed with nitric acid or 'steam cleaned' with boiling concentrated nitric acid in closed systems.

The materials used here are:

4.1.1 PP plastic vials, 50 mL.

4.1.2 PE plastic vials, 15 mL.

4.1.3 PP plastic vials, 20 mL.

4.2 Other material

4.2.1 Sleeves of aluminium foil.

4.2.2 Empty reservoirs, 8 mL (from Amchro GmbH, Hattersheim).

4.2.3 Vacuum workstation (from Amchro GmbH, Hattersheim).

4.2.4 PTFE through-flow taps (from J.T. Baker, Deventer, NL).

4.2.5 Measuring cylinders (10 mL, 20 mL, 50 mL).

4.2.6 Variable pipettes (250; 1000 µL) and suitable pipette tips.

4.2.7 10 mL measuring cylinder.

4.2.8 Horizontal mechanical shaker.

4.2.9 Centrifugal machine, maximum g number at least 5000, with suitable centrifugal vessels (50 mL PE plastic vial).

4.2.10 UV digestion unit, e.g. 'MAUV-2000 UV-Digestion System' from Maassen GmbH (Reutlingen), with suitable quartz digestion vessels.

4.2.11 Mass spectrometers with inductively coupled plasma (ICP-MS) suitable for measurements in the mass range around 200 m/z. The resolution within this range must be at least 1 mr/z (m_r = relative mass of an isotope; z = charge number) including mass-flow controller for the nebuliser gas and if possible also for the auxiliary and cooling gas, nebuliser system with adjustable low-pulsation pump and argon of at least 99.99% purity.

4.3 Reagents

4.3.1 Water from a high-purity water system, Quality: specific resistance > 18.2 MΩ cm.

4.3.2 Nitric acid, ρ(HNO_3) > 1.39 g/mL ($\geq$ 65 %), from Merck, Darmstadt.

4.3.3 Hydrochloric acid (10 %), diluted from 4.3.2 with high-purity water (4.3.1), from Merck, Darmstadt.

4.3.4 Nitric acid, 8 M, diluted from 4.3.2 with high-purity water (4.3.1).

4.3.5 Nitric acid (3 %), diluted from 4.3.2 with high-purity water (4.3.1).

4.3.6 Hydrochloric acid, ρ(HCl) > 1.15 g/mL (from J.T. Baker, Deventer) ($\geq$ 30 %).

4.3.7 Nitric acid (15 %), diluted from 4.3.6 with high-purity water (4.3.1).

4.3.8 Hydrochloric acid (5 %), diluted from 4.3.6 with high-purity water (4.3.1).

4.3.9 Ion exchanger DOWEX 1x8, 100-200 mesh, chloride form.

4.3.10 Hydrogen peroxide, 30 %, suprapur (from Merck, Darmstadt).

4.4 Standard solutions and reference material

4.4.1 Mercury standard solution, 1000 µg/mL.

4.4.2 Rhodium standard solution, 1000 µg/mL.

4.4.3 Certified reference water, e.g. NIST 1641d.

4.4.4 Certified reference material with matrix similar to sample.

5 Test procedure

5.1 Selection and weighing of samples

For the Federal Environmental Specimen Bank (ESB) the materials generally tested for methyl mercury content are zebra mussels, mussels and roe-deer liver. The samples for testing are taken from the ESB archive. For every annual homogenate three ESB specimens are routinely tested (two replicates each, i.e. n = 6) and two ESB reference homogenates of the same matrix.

For the extraction 50 mL PE plastic vials are used. These are fitted with aluminium sleeves to ensure that the extraction is carried out in complete absence of light. For each analysis, exactly 1.0 g of the ESB homogenate sample are weighed into a light-proof vial. A suitable certified reference material is also analysed to monitor the process and determine the measurement uncertainty. Should the reference material contain higher methyl mercury contents than the sample, the quantity of material may be reduced (e.g. 0.2 g NIST 2977 mussel tissue in each case).

5.2 Extraction of samples

20 mL of the 15 % hydrochloric acid (4.3.7) is added to the material being tested and extracted overnight in the mechanical shaker (150 min^{-1}). As quality assurance measure two blank values per measurement series consisting of 20 mL hydrochloric acid (15%) only are also tested throughout the entire process. Following extraction, the samples are centrifuged (20 min at 5000 g) to separate the solid from the liquid phases. Immediately after centrifuging, the liquid phase is transferred carefully to 20 mL plastic vials because the solid remaining at the bottom re-dissolves rapidly. The supernatant thus obtained is stored in complete absence of light until the ion-exchanging process can take place (max. 2 hours storage time).

5.3 Ion-exchange process

The organic mercury is separated from the inorganic by means of the strongly basic anionic exchanger DOWEX 1x8 (in the chloride form) which consists of a polystyrene-resin matrix and functional trimethyl-ammonium groups.

Firstly, empty 8 mL-reservoirs are filled with 50 mg quartz wool, 1 g ion-exchanger material and again 50 mg quartz wool in layers. The ion-exchanger columns are then fixed to the extraction chambers fitted with PTFE flow taps. Usually 4-6 samples are prepared simultaneously. The columns are first washed with 20 mL 8 M HNO_3 and 20 mL high-purity water. With 20 mL hydrochloric acid (15 %), the ion exchanger is transferred to the chloride form, so that the chloride ions of the hydrochloric acid adhere to the tertiary amino groups. When this has taken place, the extracted centrifuged eluates are passed over the columns. The methyl mercury passes through the exchanger column and emerges as CH_3HgCl. The inorganic mercury compounds on the other hand are retained by the anion-exchanger column as chlorinated complexes ($[HgCl_4]^{2-}$) and do not pass through the exchanger material. As soon as the entire sample has passed through the column another 10 mL hydrochloric acid (15 %) is added to eluate the remaining residues of methyl mercury. The entire volume remaining for testing is thus 30 mL (reference value for assessment).

5.4 UV digestion

After the ion-exchange process, 2 mL of hydrogen peroxide are added to the methyl mercury eluates. This is then distributed in the UV digestion tubes and subjected to a 2-hour process of digestion. Digestion is necessary if the samples show distinct colouring (a sign of high matrix contamination) as this would produce signal distortions during the ICP-MS analysis. Under UV radiation, the hydrogen peroxide oxidises most of the interfering matrix, thereby producing clear slightly coloured solutions after digestion.

5.5 Determination of methyl mercury by means of ICP-MS

The digested samples are prepared for ICP-MS analysis. For the basic settings and operating

procedure, refer to the SOP for ICP-MS (Determination of Elemental Content in Environmental Samples by Inductively-Coupled Plasma Mass Spectrometry, RÜDEL ET AL., 2011).

For quantitative measurement, two methods are used to determine the mercury content of samples, blank values and reagent blank values (hydrochloric acid, 15%; 10 reagent blank values analysed per measurement series):

Calibration method: after measuring a set of calibration standards, the mercury content of the sample is determined by means of a straight calibration line (linear regression). This method is used for the blank values and reagent blank values. It can also be used if the matrix of the standard corresponds for the most part with the matrix of the analysis sample.

Standard addition: The standard addition method is a type of calibration within the actual sample matrix by gradually adding a defined quantity of the analyte. This method is used similarly to the standard DIN 32633 for the sample types of the ESB and for reference materials. In this way, it is attempted to correct existing matrix effects such as increases or decreases in signal when the matrix of the analysis sample is more complex than that of the calibration solutions.

In standard addition, the sample being analysed is divided into three equal parts. Nothing is added to the first of these. To the other two eluates, different portions of a mercury standard (50 and 100 % of the anticipated analysis value) are added. To monitor the process, the mercury content of a certified reference solution is also analysed. The samples for examination (e.g. homogenate samples from the ESB) and specimens of the reference materials and the certified reference solution are prepared in this way for analysis with the ICP-MS.

An example for calculating the spike concentrations for standard addition is shown in appendix A.

The Hg concentrations of the blank values and the reagent blank values are determined using a straight calibration line as described above. The certified mercury content of a reference solution is also measured to verify the accuracy of the process.

The treated samples are measured using the ICP-MS. ^{202}Hg is determined as the main isotope.

6 Validation of method

If the content of methyl mercury was determined by standard addition, no additional standard-addition tests are required for method validation.

To validate the method, suitable certified reference materials should therefore be analysed (selection criterion: the matrix and concentration range should be as similar as possible). The criterion of quality is a correspondence of 100 $\pm$ 20 % of the determined amount with the certified contents.

For method validation, the following process parameters should also be determined:

Selectivity / specificity: these are met if the amount measured for the reagent blank value is less than the lowest validated concentration.

Reproducibility: the reproducibility is calculated from the recovery data of the reference materials via the relative standard deviation (S_{rel}). This condition is fulfilled if the following applies: $S_{rel} < 15$ % ($n \geq 5$).

Lowest limit of measuring range: the lowest limit of the method is the calibration point for which the signal-noise ratio is no less than 6:1.

Limit of detection / limit of determination: The limit of detection is calculated from blank value analyses (according to the standard DIN 32645: blank test method, quick estimate). The limit of determination is calculated by multiplying the limit of detection by a factor of 3.

7 Documentation

For the raw data, at least the following information should be documented:

- date of processing;
- name of the operator;

- instrument settings;
- preliminary treatment of sample;
- identification of samples (e.g. ESB code).

8 Evaluation

The analysis data are evaluated by the software in the mass spectrometer. The automatic evaluation must be verified for plausibility. During evaluation, the analysis data are calculated in accordance with the mathematical corrections selected and then related to the internal standard. For the purposes of calibration, linear regression is carried out in order to calculate the slope, ordinate intercepts and the coefficient of correlation (r). The concentrations of all the analysis solutions (blank values, aqueous reference materials, extracts) are determined on the basis of the straight calibration lines.

Any dilution operations must also be taken into account in all calculations. It must be possible to reconstruct all the calculations carried out by means of the electronically stored data or printouts of all data files.

When examining extracts of solid samples, the results must refer to the soild (i.e. to the fresh mass used in case of ESB samples). For ESB samples, the fresh weight related data are converted to dry mass by using the respective water content of the sample.

The concentration of mercury in the final measurement solutions after ion-exchange is generally determined by the ICP-MS software. The further calculation of the element contents in the solid matter is done by means of the following equation:

$\omega_{ww} = V / M * \rho$

where:

ω_{ww} mass proportion of mercury given as solid e.g. as ng/g

M mass of the sample used given in mg (e.g. 1.00 g)

V volume to which replenished, given in mL (e.g. 30.0 mL)

ρ mercury concentration in the measurement solution, e.g. given in ng/mL.

If fresh mass was used, the content can be converted to refer to the dry-mass content if the water content is known:

$\omega_{dw} = \omega_{ww} * 100 / (100 – WG)$

where:

WG water content of the sample in %.

From the mercury content the concentration of methyl mercury (as cation) can be calculated by multiplying with a factor of 1.0749 (mass ratio of methyl mercury cation to mercury: 215.62/200.59).

9 Statement of Results

The results refer to the amount of solid material used (fresh mass or converted to dry mass).

All results should be stated to three significant places.

EXAMPLES: Methyl mercury (as cation) determined as Hg after hydrochloric acid extraction and separation by ion exchange: 123 ng/g (or: 12,3 ng/g, 1,23 ng/g).

Measurement results are subject to a degree of uncertainty. In the working range of a process, the measurement uncertainty increases as the concentration in the sample decreases. The degree of uncertainty of a measured value can be determined in a number of ways which are described in ‘ISO Guide to the Expression of Uncertainty in Measurement (GUM)’ (ISO, 1995) and guideline ‘Quantifying Uncertainty in Analytical Measurement’ (EURACHEM/CITAC, 2000). A practical means of determining uncertainty is the so-called Nordtest process (MAGNUSSON ET AL., 2003; calculation from replicate measurements of certified reference materials and interlaboratory comparison test results).

NOTE: For the analysis of Environment Specimen Bank samples, generally six sub-samples from one homogenate are used. The standard deviation of the average value is regarded as the measurement uncertainty of the result. The

correctness of the results is verified with the help of certified reference materials. Representative data are given in the appendix.

10 Analysis report

The following data should be documented in the analysis report:

- reference to this guideline,
- sample identity,
- concentration of methyl mercury compounds with reference to fresh or dry mass (depending on material used),
- statement of measurement uncertainty if applicable,
- data on preliminary treatment of sample and digestion,
- any deviations from this guideline.

11 Representative analysis results

Representative results of analyses are given in appendix B:

a) results of the analysis of certified reference materials,

b) results of the analysis of reference materials from the Environmental Specimen Bank,

c) results of the analysis of representative samples from the Environmental Specimen Bank.

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Appendix A: Example for a Standard Addition Calculation

The aim is to determine the content of methyl mercury compounds in zebra mussels from the site Saar/Güdingen from the year 1999. The data search in the IS UPB (information system of the Environmental-Sample Bank) yielded the following reference values:

Table A1.1: Time series, zebra mussels Saar/Rehlingen.

Year	1995	1996	1997	1998
Methyl mercury [ng/g] dry weight	46.0	24.8	32.7	31.2

For the year 1999 an approximate value of 40 ng/g methyl mercury (215.62 g/mol) is assumed. This is equivalent to an Hg content (200.59 g/mol) of 37 ng/g. The water content is 93 % (as stated in the IS UPB). When converted to ng/g fresh weight, an Hg content of 2.59 ng/g Hg is obtained. For a sample-quantity used of 1 g and a final volume of 30 mL hydrochloric acid, this corresponds to 86 ng/L.

The sample is divided into 3 parts (sample without spike, sample with first spike level, sample with second spike level) and filled up to 5 mL in each case with the parent solution shown below.

Parent solution: 2.14 µg/L (in hydrochloric acid, 15 %)

Addition of first dotation (100 µL): 43 ng/L, i.e. approx. 50 % of the anticipated value;

Addition of second dotation (200 µL): 86 ng/L, i.e. approx. 100 % of the anticipated value.

The ^{202}Hg content of the specimens thus obtained is determined by means of ICP-MS.

For calibration purposes, an Hg parent solution (in hydrochloric acid, 15%) with a concentration of 1 µg/L is prepared. For calibration, the following concentration levels have to be prepared: 0, 10, 25, 50 and 75 ng/L Hg.

By using the straight calibration line derived from linear regression, it is possible to determine the Hg content of the reagent blank values, the blank values and the certified reference solution.

Appendix B: Representative Analysis Results

a) Results for certified reference materials (dry mass).

Reference material	Certified content	Recovery	Remarks
Series A (2005)			
NIST 1641d	159 ng/L	89.3 ± 13.3% (n=10)	CRM solution
NIST 2977	36.2 ng/g	84.8 ± 9.4% (n=5)	CRM mussels
Series B (2006)			
NIST 1641d	159 ng/L	84.6 ± 5.7% (n=12)	CRM solution
NIST 2977	36.2 ng/g	82.8 ± 7.0% (n=6)	CRM mussels

b) Results for ESB reference materials (dry mass; retrieved from the information system of the ESB).

USB Reference material	Content (from IS ESB)	Recovery
4110/0/0086/07102/0 (blue mussels)	166.5 ng/g	95.4 ± 15.4 % (n =5)
3010/0/1198/10305/0 (zebra mussels)	69.8 ng/g	98.2 ± 7.9% (n=6)

c) Examples of results from the analysis of representative ESB samples (dry mass).

Sample	ESB code	Content (from IS ESB)
Blue mussels, Darßer Ort 2005	4110/0/0005/06103/0	11.7 ± 1.4 ng/g (n =5)
Blue mussels, Eckwarderhörne 2005	4110/0/0005/07302/0/	68.3 ± 8.0 ng/g (n=6)
Zebra mussels, Barby 2004	3010/0/1104/10205/0	96.0 ± 11.2 ng/g (n=6)