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Water quality — Determination of alkylmercury compounds in water — Method using gas chromatography-mass spectrometry (GC-MS) after phenylation and solvent extraction

Qualité de l'eau — Détermination des composés alkyl mercure dans l'eau — Méthode par chromatographie gazeuse et spectrométrie de masse (CG-SM) après phénylation et extraction par solvant

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document specifies a method for the determination of alkylmercury compounds in water by gas chromatography-mass spectrometry (GC-MS) after phenylation and solvent extraction.

Alkylmercury has high toxicity that causes Minamata disease in the heavy exposure as discovered at Minamata City in Japan in 1956. Methylmercury in wastewater from an acetaldehyde acetic acid manufacturing plant was identified as a causative substance. Subsequent investigation revealed that ethylmercury poisoning has a similar toxic effect as methylmercury. Japanese government set an effluent standard and an environment standard for alkylmercury.

Minamata Convention on Mercury was adopted by over 140 countries in 2013 for prevention of global environmental pollution and health damage caused by mercury, and entered into force in 2017. The convention states that each party shall identify the relevant point source categories and take measures including the set of release limit values and the use of best available techniques and best environmental practices. It should be noted that the released inorganic mercury is partially converted to alkylmercury by biochemical processes of microorganism in water and sediment. Alkylmercury is concentrated in biota through food chain, and consequently the risk to higher organism increases.

This document will be beneficial to evaluate the risk of alkylmercury from water and to control the anthropogenic releases of alkylmercury from the relevant point sources.

Water quality — Determination of alkylmercury compounds in water — Method using gas chromatography-mass spectrometry (GC-MS) after phenylation and solvent extraction

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies a method for the determination of alkylmercury compounds in filtered water samples by gas chromatography-mass spectrometry after phenylation and solvent extraction.

This method is applicable to determination of individual methylmercury (MeHg) and ethylmercury (EtHg) compounds in surface water and waste water.

The method can be applied to samples containing 0,2 µg/l to 10 µg/l of each compound as mercury mass. Depending on the matrix, the method may also be applicable to higher concentrations after suitable dilution of the sample or reduction in sample size.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Principle

Alkylmercury compounds dissolved in a water sample are phenylated with sodium tetraphenylborate after adjusting the sample solution at pH 5,0 with acetate buffer. The phenylated mercury compounds are extracted from the water samples into toluene by liquid-liquid extraction. An internal standard is added to the sample solution prior to pH-adjustment of sample preparation. The phenylated mercury compounds in the toluene extracts are determined by gas chromatography-mass spectrometry (GC-MS).

5 Interferences

5.1 Interferences with sampling, sample storage and sample preparation

Potential sources of mercury or alkylmercury contamination during sampling, sample storage and sample preparation include: labware, containers, sampling equipment, reagents, reagent water, atmospheric dirt and dust, and human contact.

Only fluoropolymer or borosilicate glass containers shall be used for sample storage because mercury vapor can diffuse in or out of sample solution, if other materials are used.

Apparatus or parts which may come into contact with either water sample or the extract shall be non-metallic and free from target substances to be determined and interfering substances. All apparatus and labware shall be cleaned using the cleaning procedure in this method.

Phenylation and solvent extraction are prone to be interfered with large amounts of coexisting substances. For the majority of natural water samples, this type of interference should not be significant, but severe interference may be found in some waste water samples which contain high concentration of coexisting substances. Some organosulfur compounds (e.g. L-cysteine) binding alkylmercury also interfere with the phenylation. The user should know the level of coexisting substances on typical water samples using appropriate methods and if severe interferences are indicated, the level of interferences should be assessed by recovery tests with spiking a standard solution of alkylmercury into water samples.

If the coexisting substances in a water sample are precipitated with tetraphenylborate during phenylation step, the precipitates may interfere with the solvent extraction procedure causing problems in phase separation. For example, potassium of high concentration (> several hundred mg/l) in water precipitates tetraphenylborate, that leads to interfere with the phase separation. In these cases, filter the water sample through a glass fibre filter (7.2.2), and then add a new solution of sodium tetraphenylborate and toluene.

5.2 Interferences with GC-MS

Substances that co-elute with the target alkylmercury or the internal standard may interfere with the determination. These interferences may lead to incompletely resolved signals and may, depending on their magnitude, affect accuracy and precision of the analytical results. Non-symmetrical peaks and peaks broader than the corresponding peaks of the reference substance suggest interferences.

Interferences may also be caused by carry-over contamination mainly from the injection system of the GC, especially when analysing a sample with low concentration after a sample with much higher concentration of alkylmercury. A memory test, by injecting toluene, is useful to check the extent of carry-over.

6 Reagents and standards

Unless otherwise indicated, reagents of purity grade "for analysis" or "for residue analysis" are used as reagents.

6.1 Water, grade 1, as specified in ISO 3696.

6.2 Hydrochloric acid, c(HCl) = 10 mol/l, ultra-pure grade.

6.3 Acetic acid, $c(CH_3COOH)$ = 17 mol/l, ultra-pure grade.

6.4 Sodium hydroxide solution, c(NaOH) = 3 mol/l, ultra-pure grade.

6.5 Acetate buffer solution

An aliquot of 11,5 ml of acetic acid (6.3) and 42,5 ml of sodium hydroxide solution (6.4) is dissolved in water (6.1) to give a final volume of 1,0 l. The final concentration of the acetate is 0,2 mol/l.

6.6 Sodium tetraphenylborate solution, ρ = 20 g/l.

Sodium tetraphenylborate reagent is of purity grade for gas chromatographic analysis, or equivalent. Two grams of the reagent is dissolved in water (6.1) to give a final volume of 100 ml. The solution should be immediately used within 1 d after the preparation because the phenylation activity reduces significantly after this time.

6.7 Toluene, $C_6H_5CH_3$.

6.8 Methanol, CH_3OH.

6.9 Sodium sulfate, Na_2SO_4, anhydrous, powdered.

6.10 Methylmercury chloride, CH_3HgCl.

6.11 Ethylmercury chloride, C_2H_5HgCl.

6.12 2,4,6-trichloroanisole-d3, $C_7H_2D_3Cl_3O$.

6.13 Poly(ethylene glycol) 300 solution, PEG300, ρ = 100 g/l.

Dissolve 1 g of poly(ethylene glycol) 300 of reagent grade in 10 ml of toluene (6.7) in a stoppered test tube (7.5).

6.14 Stock solutions of methylmercury chloride and ethylmercury chloride, ρ_{Hg} = 1 000 mg/l.

Dissolve approximately 0,125 g, accurately weighed, of methylmercury chloride or 0,132 g, accurately weighed, of ethylmercury chloride in 100 ml of methanol (6.8) or reagent water (6.1) containing 5 ml/l acetic acid (6.3) and 2 ml/l HCl (6.2) in a fluoropolymer bottle. Each solution should contain approx. 1 000 mg/l CH_3Hg or C_2H_5Hg as Hg. It is also recommended to use commercially available certified standard solutions of methylmercury chloride or ethylmercury chloride.

6.15 Standard solutions of alkylmercury, ρ_{Hg} = 10 mg/l or 1 mg/l.

Dilute 100 µl or 10 µl of the stock solution (6.14) to 10 ml of methanol (6.8) or reagent water (6.1) containing 5 ml/l acetic acid (6.3) and 2 ml/l HCl (6.2). Each solution contains 10 mg/l or 1 mg/l CH_3Hg or C_2H_5Hg as Hg. It was reported that the amount of the alkylmercury in the standard solution using acetic acid and HCl has been maintained over a year when stored in a fluoropolymer bottle in a refrigerator (see Reference [2]).

6.16 Reference solutions of alkylmercury for calibration

Prepare a minimum of five reference solutions with different concentrations for calibration. Pour 100 ml of reagent water (6.1) in a narrow-neck flat-bottomed flask (7.3) and add stepwise from 10 µl to 100 µl

of the alkylmercury standard solution (6.15) directly into the water using a microliter syringe (7.7) without contacting it on the wall of the flask. Put the stopper on the flask and stir the solution gently. The concentrations of alkylmercury are adjusted for the calibration from 0,2 µg/l to 10 µg/l as Hg.

6.17 Check solutions of phenylated alkylmercury for performance of GC-MS

Treat the reference solution of alkylmercury at 10 µg/l (6.16) using the procedure as outlined in 9.1. The final concentration in the solvent extract should be 200 µg/l of phenylated alkylmercury species due to the preconcentration factor of the liquid-liquid extraction employed. Dilute the final extracts with toluene (6.7) at an appropriate ratio to give the concentrations of phenylated alkylmercury ranging between 4 µg/l and 200 µg/l as Hg. Add 2 µl of PEG300 solution (6.13) in the proportion of 1 ml of toluene. The addition of PEG300 is required to ensure good chromatographic behaviour of phenylated alkylmercury. These solutions are used for checking the performance of the GC-MS, such as sensitivity, linearity of calibration, and resolution of target peaks before conducting an analysis of water samples.

6.18 Internal standard solution, ρ = 40 mg/l.

Weigh 10 mg of 2,4,6-trichloroanisole-d3 (6.12) in a 10 ml volumetric flask and make up to the mark with methanol. The standard solutions that are commercially available can also be used. Dilute this solution in the ratio of 1:25 with methanol.

6.19 Operating gases for GC-MS, helium, purity ≥999,99 mmol/mol.

7 Apparatus and materials

7.1 Sample collection bottles, fluoropolymer or borosilicate glass, of capacity 125 ml to 1 000 ml, with such materials or fluoropolymer-lined cap.

New bottles should be cleaned by heating from 65 °C to 75 °C in 4 mol/l HCl for at least 48 h. After cooling, they are rinsed three or more times with reagent water, filled with reagent water containing 4 ml/l HCl (6.2) and capped, and placed in a plastic box until use. Rinse the bottles with reagent water just prior to use for sampling. Bottle blanks should be analysed as described in 9.5 to verify the effectiveness of the cleaning procedures.

7.2 Filter

7.2.1 Filter for sampling, cellulose acetate or cellulose nitrate, of pore size 0,45 µm.

7.2.2 Filter for solvent extraction, borosilicate glass fibre, diameter of fibres 0,75 µm to 1,5 µm.

7.3 Narrow-neck flat-bottomed flasks, borosilicate glass, of capacity 150 ml to 200 ml, with glass stoppers, used for phenylation and solvent extraction.

7.4 Volumetric pipettes, capacity 100 ml, used for measuring the volume of water sample with ±0,08 ml tolerance.

7.5 Stoppered test tubes, capacity 10 ml, used for pre-examination to adjust the pH of the buffered solutions (9.1.1) or for dehydration of the extracts (9.1.3).

7.6 Vials for GC-MS, made of amber glass, capacity 2 ml, with fluoropolymer-lined screw-cap.

7.7 Microlitre syringe, made of borosilicate glass.

7.8 Disposable Pasteur pipettes, made of borosilicate glass.

7.9 Balances, analytical type capable of weighing 0,1 mg.

7.10 pH meter, with combination glass electrode.

7.11 Magnetic stirrer, with PTFE-coated (PTFE = polytetrafluoroethylene) stirring bar of suitable size.

7.12 Gas chromatograph mass spectrometer (GC-MS), with electron impact ionization.

An example of operating condition of GC-MS is given in Annex A.

7.13 GC column

The column shall be capable of resolving the phenylated alkylmercury and inorganic mercury(II) compounds (phenylmethylmercury, phenylethylmercury and diphenylmercury) listed in Table 1. Examples of the columns are shown in Annex A.

Table 1 — Phenylated alkylmercury and inorganic mercury(II) compounds and internal standard and selected diagnostic ions for identification and quantification in mass spectrometric detection

Name of mercury species in water samples	Name of mercury compounds after phenylation	Molecular formula after phenylation	Selected ions for identification *m/z*	Selected ions for quantification *m/z*
Methylmercury	Phenylated methylmercury	$CH_3HgC_6H_5$	200, 202, 217, 279, 292, 294	292, 294
Ethylmercury	Phenylated ethylmercury	$C_2H_5HgC_6H_5$	200, 202, 231, 279, 306, 308	306, 308
Inorganic mercury(II)	Diphenylmercury	$C_6H_5HgC_6H_5$	200, 202, 279, 354, 356	354, 356
2,4,6-trichloroanisole-d3 (internal standard)		$C_7H_2D_3Cl_3O$	213, 215	213, 215

8 Sample collection, preservation and storage

Sample bottles should be stored in clean polyethylene bags until analysis. Samples are collected into rigorously cleaned fluoropolymer or borosilicate glass bottles as specified in ISO 5667-1 and ISO 5667-3 and References [2] and [3]. Collected samples are filtered through a 0,45 µm filter (7.2.1) and preserved at about pH 1,4 by adding an appropriate amount of HCl (6.2), which can be changed depending on the acidity of the sample, e.g. 4 ml of HCl per litre of neutral sample. Samples may be shipped to the laboratory unpreserved if they are kept dark and maintained at 0 °C to 4 °C from the time of collection until preservation. The samples shall be acid-preserved within 48 h of sampling. Methylmercury in acid-preserved samples is stable for at least six months, if kept dark and cool (Reference [2]).

9 Procedure

9.1 Sample preparation

9.1.1 pH-adjustment of water sample

Measure 100 ml of the acid-preserved water sample with a volumetric pipette (7.4) and transfer into a narrow-neck flat-bottomed flask (7.3). The exact volume of the water sample can also be calculated from its weight and density.

Following sample transfer, neutralize the acid-preserved water sample by adding an appropriate amount of NaOH solution (6.4), add 5 ml of acetate buffer solution (6.5) and 5 µl of an internal standard solution (6.18) using a microliter syringe (7.7) without contacting it on the wall of the flask and

adjust the pH of the solutions with diluted HCl or NaOH solution at pH 5,0 ± 0,1. To minimize mercury contamination, do not dip a pH glass electrode into the sample; take a small aliquot of the water sample with a clean pipet to a test tube (7.5) and examine the pH for estimating required amounts of acid or alkali for pH adjustment. In the separate pH test, record the volume used for pH test to calculate exact sample volume for phenylation. It is also recommended to use a pH meter which can measure a sample with a small volume (e.g. 0,1 ml) by placing the solution onto the flat sensor in a measuring scoop.

9.1.2 Phenylation and solvent extraction

Add 1 ml of sodium tetraphenylborate solution (6.6). Cover the flask with stopper and swirl gently to mix.

Add 5 ml of toluene and place a magnetic stirring bar in the flask and stir the water sample vigorously using a magnetic stirrer (7.11) for about 60 min at room temperature, and then allow to stand for about 10 min.

Pour reagent water (6.1) gently along the inside wall of the flask until the toluene layer rises to narrow-neck part, and transfer the toluene layer using a disposable Pasteur pipette (7.8) to a stoppered test tube (7.5).

9.1.3 Dehydration of toluene extract

To remove water from the extract, add 2 g of sodium sulfate (6.9) to the toluene extract in the stoppered test tube (7.5).

9.2 Preparation of samples for GC-MS

Transfer a supernatant aliquot (approx. 1 ml) of the dehydrated toluene to a vial for GC-MS (7.6) and add 2 µl of PEG300 solution (6.13). The addition of PEG300 is required to deactivate the active site in the injection port and the column and to ensure good chromatographic behaviour of phenylated alkylmercury especially at low concentrations.

9.3 Optimization of operating condition for GC-MS

Optimize the operating conditions of the GC-MS system in electron impact ionization mode in accordance with the manufacturer's instructions.

If setting up the method for the first time, check retention time (RT) and identity of each single compound carefully. It is recommended that each phenylated alkylmercury be single-injected for checking retention time and/or mass spectrum (examples of mass chromatograms and mass spectra are shown in Annex B).

Determine the appropriate GC oven temperature programme experimentally during method development and validation. An example of operating conditions and selected ions for identification and quantification is given in Annex A and Table 1.

9.4 Identification of individual substances with GC-MS

Identify individual substances by comparing the retention times and relative intensities of the diagnostic ions (Table 1) of sample with those of the calibration standard (6.16). It is necessary to use specific pair of ions for identification and quantification of each resolved peak.

Individual substances are regarded as identified in the sample if:

— the relative or the absolute sample component retention time measured in the selected ion current chromatogram matches the relative or absolute retention time of the authentic substance within ±0,2 % (or a maximum of ±6 s) in the chromatogram of corresponding internal standard or those of the latest reference substances, measured under identical conditions, and

— at least two selected identification masses (Table 1) occur at the substance-specific retention time, and

— the complete mass spectra of the background corrected reference substances match the spectra present at the relevant retention time in the chromatogram of the water sample, also background corrected or

— the relative intensities of all selected diagnostic ions observed for samples shall match the abundance observed for reference substances to within 25 %.

9.5 Blank tests

Check that the instruments and reagents remain in good condition by carrying out regular blank tests. To conduct the blank tests, prepare and analyse 100 ml of reagent water (6.1) in the same way as the sample. Procedural blank tests should be carried out with each batch of samples (a maximum of 10 samples).

If unusual blank values occur, find the reason for this by systematic investigations, so that the source of contamination can be eliminated.

10 Calibration

10.1 General requirements

For routine analysis, only a calibration with internal standards shall be applied.

Determine the linear working range using at least five measuring points of different concentration (see ISO 8466-1).

The calibration function for a substance is valid only for the measured concentration range. Additionally, the calibration function depends on the condition of the instrument and shall be checked regularly.

Table 2 gives an explanation of the subscripts used in the formulae and in the following text.

Table 2 — Explanation of subscripts

Subscript	Meaning
i	Identity of the target substance
e	Calibration step
g	Sample analysis step
j	Consecutive figure for pairs of values
is	Internal standard

10.2 Performance test of GC-MS

For daily check of the performance of GC-MS, (such as background level, sensitivity, linearity of calibration, and resolution of target peaks), use toluene and the check solutions of phenylated alkylmercury (6.17). For each target substance, establish a calibration function and check the sensitivity by measurement of two points; it is practicable to include in one step all target substances.

It is also recommended to carry out the performance test of the GC-MS system during analysis of water samples by injecting the check solutions (6.17) regularly. If any deterioration in the performance are detected, e.g. the sensitivity differs by more than 20 % from the latest one, find the reason for this by systematic investigations, so that the cause for deterioration can be eliminated. Prepare a new calibration curve if the GC-MS conditions change or if the sensitivity differs by more than 20 % even after systematic investigations.

The injection volume in the performance test shall be the same as that in the analysis of water samples.

10.3 Calibration with internal standard

10.3.1 General requirement

The use of an internal standard helps to minimize errors that may occur during injection step to GC as well as errors caused in sample preparation steps such as phenylation, solvent extraction and dehydration of the extract. As an internal standard, a compound with the same physicochemical properties as those of the analytes is preferable. From this point of view, Hg–isotope enriched methylmercury chloride, employed in an alternative derivatization (propylation described in Annex C), is a promising candidate. However, in phenylation, it is not practical to use this reagent because of increased uncertainty in getting the isotope ratio from the complex mass spectrum due to larger number of ^{13}C atoms of the phenyl group, along with the limited availability and the high cost of the Hg-isotope enriched reagent.

If a compound, which is not subjected to phenylation, is used as an internal standard, interference on phenylation cannot be compensated. However, even in such cases, satisfactory results can be obtained for the majority of natural water samples, since the concentrations of coexisting substances are relatively low and the interference to phenylation are not significant.

For samples containing large amounts of coexisting substances and if severe interference to phenylation is suspected, the level of interference should be assessed by recovery tests with spiking alkylmercury and internal standard into the water samples. If the recovery rate is out of the range described in 10.4 and 11.3, the analytical values are not accepted.

From considering the facts mentioned above, 2,4,6-trichloroanisole-d3 is typically used as an internal standard for correcting the variations of volume in the solvent extraction and the GC injection steps and the drift of sensitivity of mass spectrometer in this method, but does not correct for the phenylation stage.

10.3.2 Procedure of calibration

Measure 100 ml of reagent water (6.1) and reference solutions of alkylmercury (6.16) with a volumetric pipette (7.4) and transfer into a narrow-neck flat-bottomed flask (7.3), and then follow the procedure described in Clause 9. The same amounts of HCl, NaOH and acetate buffer solutions as those for water sample shall be used (within the practicality of the method). The mass concentration of the internal standard shall be almost equal for both calibration and sample measurement.

Plot the values of the ratio $y_{i,\mathrm{e},j} / y_{is,\mathrm{e},j}$ (integration units e.g. for peak area) for each substance i on the ordinate and the associated ratio of the mass concentrations $\rho_{i,\mathrm{e},j} / \rho_{is,\mathrm{e},j}$ on the abscissa.

Determine the linear regression function using the corresponding pairs of values $y_{i,\mathrm{e},j}/y_{is,\mathrm{e},j}$ and $\rho_{i,\mathrm{e},j}/\rho_{is,\mathrm{e},j}$ of the measured series in accordance with Formula (1):

$$\frac{y_{i,\mathrm{e}}}{y_{is,\mathrm{e}}} = m_{i,is,\mathrm{e}} \frac{\rho_{i,\mathrm{e}}}{\rho_{is,\mathrm{e}}} + b_{i,is,\mathrm{e}} \tag{1}$$

where

$y_{i,\mathrm{e}}$ is the measured value of substance i during calibration, a variable dependent on $\rho_{i,\mathrm{e}}$, whose unit depends on the evaluation, e.g. area unit;

$y_{is,\mathrm{e}}$ is the measured value of internal standard (*is*) during calibration, whose unit depends on the evaluation, e.g. area unit; all reference solutions contain equal concentrations of the internal standard;

$\rho_{i,\mathrm{e}}$ is the independent variable mass concentration of substance i in the reference solution, in micrograms per litre, µg/l;

$\rho_{is\,e}$ is the independent variable mass concentration of internal standard (*is*) in the reference solution, in micrograms per litre, µg/l;

$m_{i,is,e}$ is the slope of the calibration curve from $y_{i,e}/y_{is,e}$ as a function of the mass concentration ratio $\rho_{i,e}/\rho_{is,e}$, often called the response factor;

$b_{i,is,e}$ is the ordinate intercept of the calibration, the unit depends on the evaluation.

10.4 Spike recovery test of target substances

When analysing a sample susceptible to matrix effect or an unknown sample, a recovery test should be carried out by analysing the sample, spiked sample with target substance, blank and standard.

For example, add 10 µl of 10 mg/l alkylmercury standard solution (6.15) to 100 ml of a water sample and reagent water, respectively, conduct sample preparation and analyse them, as well as a non-spiked water sample and non-spiked reagent water as given in Clause 9.

Calculate the recovery A_i in accordance with Formula (2):

$$A_i = 100 \times \frac{\left(y_{i,\mathrm{spk}} - y_{i,\mathrm{nonspk}}\right)}{\left(y_{i,\mathrm{std}} - y_{i,\mathrm{blk}}\right)} \tag{2}$$

where

$A_{i,}$ is the recovery of substance *i* in percent, %;

$y_{i,\mathrm{spk}}$ is the measured value of substance *i* in spiked water sample, e.g. area unit;

$y_{i,\mathrm{nospk}}$ is the measured value of substance *i* in non-spiked water sample, e.g. area unit;

$y_{\mathrm{i,std}}$ is the measured value of substance *i* in spiked reagent water, e.g. area unit;

$y_{i,\mathrm{blk}}$ is the measured value of substance *i* in non-spiked reagent water, e.g. area unit.

Recovery should preferably be in the range from 70 % up to 120 %. A lower or higher value indicates an insufficient efficiency of phenylation and extraction or co-extraction of interfering substances for mass spectrometric measurement, respectively. When the recovery falls outside the range, dilute the water sample with an appropriate factor, e.g. 5 times, and repeat the recovery test. If the recovery for the diluted sample falls within the range, then the water sample used for sample preparation (9.1) shall also be diluted with either this dilution factor or the dilution factor for recovery of internal standard (11.3), whichever is greater. If the recovery still falls outside the range even for the sample diluted more than 10 times, stop the analysis and report the variation on the test report.

11 Calculation

11.1 Calculation of results after calibration with internal standards

Determine the result for each sample using the calibration curve prepared as described in 10.3.

Calculate the mass concentration $\rho_{i,g}$ of target substance *i* in accordance with Formula (3) after solving Formula (1):

$$\rho_{i,g} = \left(\frac{y_{i,g}}{y_{is,g}} - b_{i,is,e} \right) \cdot \frac{\rho_{is,g}}{m_{i,is,e}} \tag{3}$$

where

$y_{i,g}$ is the measured value of target substance *i* in the sample, e.g. area unit;

$y_{is,g}$ is the measured value of internal standard (*is*) in the sample, e.g. area unit;

$\rho_{i,g}$ is the mass concentration of target substance *i* in the sample, in micrograms per litre, µg/l;

$\rho_{is,g}$ is the mass concentration of internal standard (*is*) in the sample, in micrograms per litre, µg/l;

$b_{i,is,e}$, $m_{i,is,e}$ see Formula (1).

11.2 Treatment of results lying outside the calibration range

If the concentrations of the target substance in the sample lie outside of the range of the calibration curve, use a smaller volume of sample for the phenylation and extraction or dilute the final extract by a suitable factor with toluene.

11.3 Quality checks for internal standardization

Determine recovery rates of the internal standard after optimizing the extraction and concentration procedure from Formula (4). The recovery of the internal standard shall be between 70 % and 120 % for the internal standard batch to be considered acceptable. When the recovery falls outside the range, dilute the water sample with an appropriate factor, e.g. 5 times, and repeat the recovery test. If the recovery for the diluted sample falls within the range, then the water sample used for sample preparation (9.1) shall also be diluted with either this dilution factor or the dilution factor for spike recovery (10.4), whichever is greater. If the recovery still falls outside the range even for the sample diluted more than 10 times, stop the analysis and report the variation on the test report.

$$R_{is} = 100 \times \frac{\rho_{is,g} \cdot V_s}{\rho_{is} \cdot V_{is}} \quad (4)$$

where

R_{is} is the percent recovery of internal standard (*is*) from the sample;

$\rho_{is\ g}$ see Formula (3);

ρ_{is} is the mass concentration of internal standard (*is*) in the internal standard solution added to the sample, in micrograms per litre, µg/l;

V_s is the volume of the sample, expressed in litres, l;

V_{is} is the volume of the internal standard solution added to the sample, expressed in litres, l.

12 Expression of results

The analysis results obtained when applying this document are subject to a measurement uncertainty that is to be considered in the interpretation of the results (see Annex E).

The mass concentration of the individual alkylmercury in micrograms of mercury per litre (µg/l as Hg) should be reported with two significant figures.

EXAMPLES

Methylmercury 0,31 µg/l as Hg

Ethylmercury 1,4 µg/l as Hg

13 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 21863:2020;

b) all information necessary for the complete identification of the sample;

c) the sample storage protocol;

d) the results obtained for the individual compounds, expressed in accordance with Clause 12;

e) the recovery of internal standard;

f) details of any deviations from this procedure and of all circumstances which may have influenced the results;

g) the date of analysis.

Annex A
(informative)

Example of operating condition of GC-MS

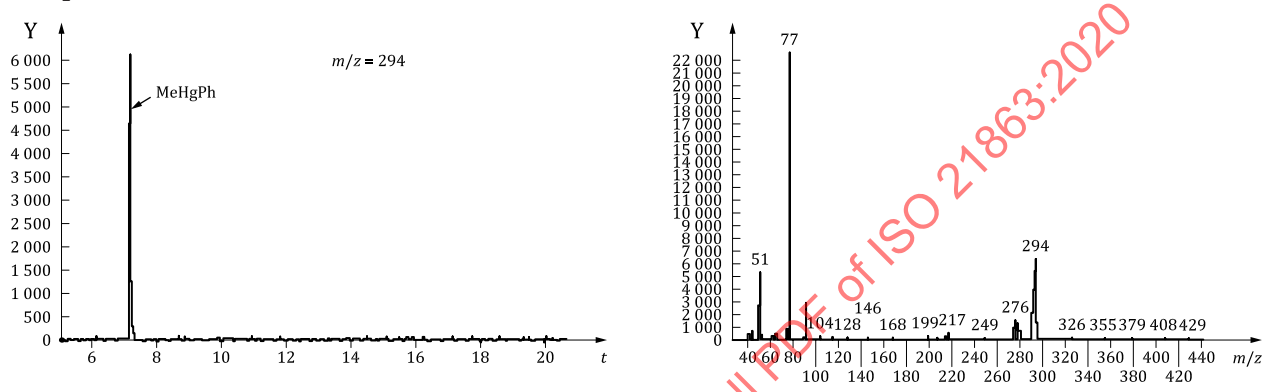
GC	
Column[a]	100 % dimethylpolysiloxane, length: 30 m, diameter: 0,25 mm, film thickness: 0,25 µm, e.g. Rxi-1ms or DB-1ms.
Column temperature	90 °C (1 min) → 8 °C/min → 220 °C → 30 °C/min → 280 °C (3 min)
Carrier gas	He, 999,99 mmol/mol; constant linear velocity mode, 40,0 cm/s (initial gas flow rate: 1,17 ml/min)
Sample Injection mode	Splitless, pulsed injection (also known as high pressure injection) at 250 kPa for 1,5 min
Injection volume	1 µl
Injection temperature	280 °C
MS	
Type	Quadrupole
Interface temperature	280 °C
Ionization	EI 70 eV
Ion source temperature	230 °C
Measurement mode	SIM
Monitored ion (*m*/*z*)	MeHgPh: 292, 294 EtHgPh: 306, 308 HgPh2: 354, 356 2,4,6-trichloroanisole-d3 (internal standard): 213, 215

[a] Rxi-1ms and DB-1ms are trade names of a product supplied by Restek Corporation and Agilent Technologies, respectively. As an alternative to the column of 100 % dimethylpolysiloxane, a 5 % polydiphenyl- / 95 % polydimethylsiloxane column may be used (length: 30 m, diameter: 0,25 mm, film thickness: 0,25 µm; e.g. Restek Rxi-5ms or Agilent DB-5ms). These examples are given only as information for the users of this document and do not constitute an endorsement by ISO of these products.

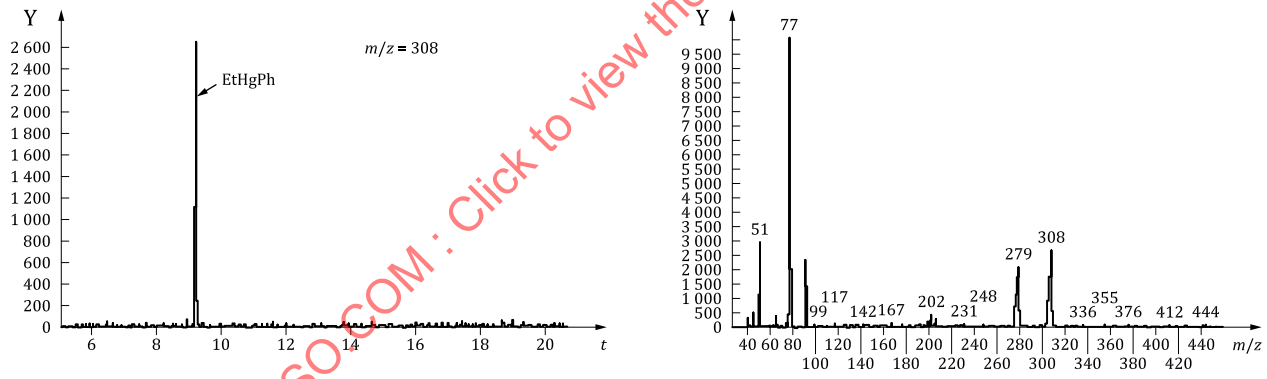
Annex B
(informative)

Examples of mass chromatograms and mass spectra of phenylated alkylmercury by GC-MS

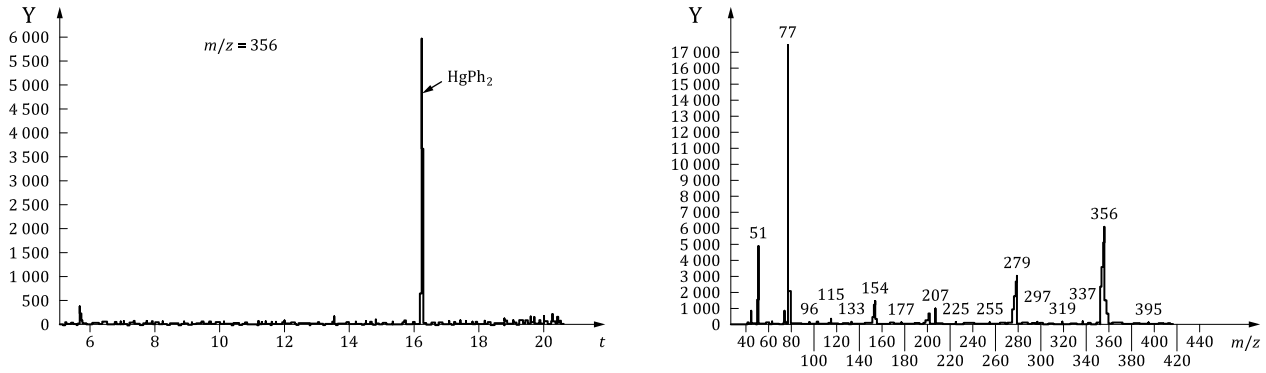
Example 1



a) Methylmercury



b) Ethylmercury



c) Inorganic mercury

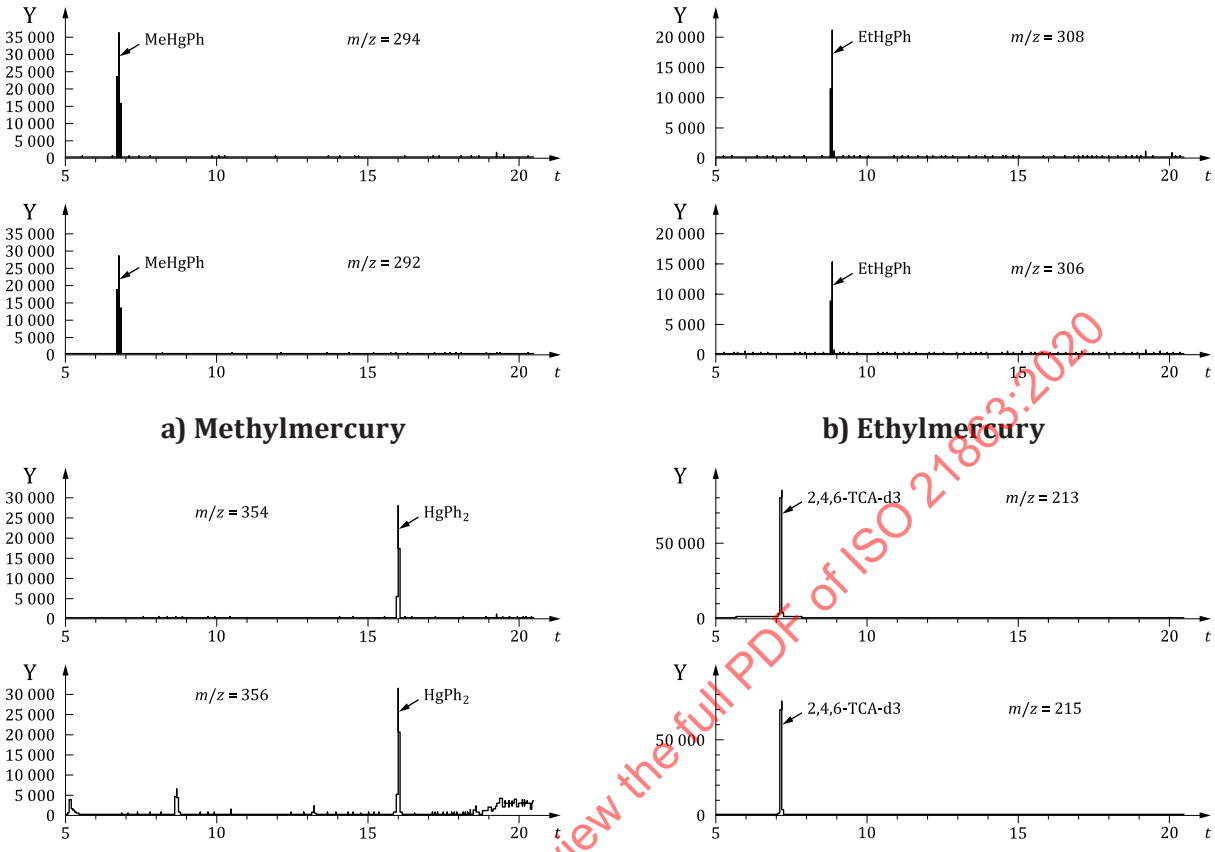
Key

t retention time (min)

Y signal intensity

Figure B.1 — Examples of mass chromatograms and mass spectra of phenylated compounds by GC-MS

Example 2



Key

t retention time (min)

Y signal intensity

Figure B.2 — Examples of mass chromatograms of phenylated compounds and an internal standard compound by GC-MS

Annex C
(informative)

The use of sodium tetrapropylborate as an alternative derivatizing agent[4]

As an alternative to the sodium tetraphenylborate reagent (6.6), sodium tetrapropylborate may be used as a derivatizing agent:

Sodium tetrapropylborate, ρ = 100 g/kg in tetrahydrofuran (THF).

Sodium tetrapropylborate reagent is of purity grade for gas chromatography analysis, or equivalent. 5 g of the reagent is dissolved in THF to give a final weight of 50 g. The solution can be stored for at least one month at a temperature of –18 °C in the dark. The longer storage time in comparison to the sodium tetraphenylborate solution results from the higher concentration of the solution (100 g/kg) and stabilization by THF.

The procedure for sample preparation as given in Clause 9 is the same for the derivatization agent sodium tetrapropylborate as for sodium tetraphenylborate.

As an alternative to the 2,4,6-trichloroanisole-d3 solution (ρ = 40 mg/l) (see 6.18), ^{202}Hg (or another isotope)- enriched methylmercury chloride solution (ρ_{Hg} = 5 mg/l) may be used as an internal standard. Prepare the solution according to the procedure given in 6.14 and 6.15.

Regarding 7.13:

The selected diagnostic ions for identification and quantification have to be adapted to the molecular weight of propylated alkylmercury compounds. Possible ions are listed in Table C.1. If different Hg isotopes are used, the mass for identification and quantification have to be recalculated.

Table C.1 — Propylated alkylmercury and inorganic mercury(II) compounds and selected diagnostic ions for identification and quantification in mass spectrometric detection

Name of mercury species in water samples	Name of mercury compounds after propylation	Molecular formula after propylation	Selected ions for identification *m/z*	Selected ions for quantification *m/z*
Methylmercury	Propylated methylmercury	$CH_3HgC_3H_7$	200, 202, 217, 245, 258, 260	258, 260
Ethylmercury	Propylated ethylmercury	$C_2H_5HgC_3H_7$	200, 202, 231, 245, 272, 274	272, 274
Inorganic mercury (II)	Dipropylmercury	$C_3H_7HgC_3H_7$	200, 202, 243, 286, 288	286, 288
^{202}Hg-enriched methylmercury (internal standard)	Propylated methylmercury	$CH_3{}^{202}HgC_3H_7$	202, 217, 245, 260	260