
**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

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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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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.

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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.

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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

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, $c(\text{HCl}) = 10 \text{ mol/l}$, ultra-pure grade.

6.3 Acetic acid, $c(\text{CH}_3\text{COOH}) = 17 \text{ mol/l}$, ultra-pure grade.

6.4 Sodium hydroxide solution, $c(\text{NaOH}) = 3 \text{ mol/l}$, 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, $\rho = 20 \text{ g/l}$.

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, $\text{C}_6\text{H}_5\text{CH}_3$.

6.8 Methanol, CH_3OH .

6.9 Sodium sulfate, Na_2SO_4 , anhydrous, powdered.

6.10 Methylmercury chloride, CH_3HgCl .

6.11 Ethylmercury chloride, $\text{C}_2\text{H}_5\text{HgCl}$.

6.12 2,4,6-trichloroanisole-d3, $\text{C}_7\text{H}_2\text{D}_3\text{Cl}_3\text{O}$.

6.13 Poly(ethylene glycol) 300 solution, PEG300, $\rho = 100 \text{ g/l}$.

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, $\rho_{\text{Hg}} = 1\,000 \text{ mg/l}$.

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 $\text{C}_2\text{H}_5\text{Hg}$ 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, $\rho_{\text{Hg}} = 10 \text{ mg/l}$ 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 $\text{C}_2\text{H}_5\text{Hg}$ 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, $\rho = 40$ 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 $\geq 999,99$ 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 $\pm 0,08$ 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.