Development of a Solid Phase Extraction-based Method for Quantitative Analysis of Methylmercury in Soil and Sediment

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Abstract

Methylmercury (MeHg) pollution is currently widespread in paddy soil and sediment, posing a health hazard risk during the harvesting of rice grains. However, to date, a simple and universal method for quantifying MeHg in the soil is unavailable. Therefore, we aimed to develop a solid-phase extraction-based method using gas chromatography mass spectrometry (GC-MS). In this study, MeHg was purified from the soil matrix using optimized solid-phase extraction; our method reduced the use of organic solvents and did not require harmful reagents such as alkaline solution and toluene. The limit of quantification in the sample was determined to be 7.5 ng/g. The MeHg recovery in reference samples was 96.2–102.6%, and intra- and inter-assay coefficients of variation were 3.4–7.1% and 4.3–7.1%, respectively, indicating high validation performance. Furthermore, the MeHg levels of the five tested reference samples were determined to be 64.1 ng/g or not detected, and these levels were well below the Japanese regulatory criteria. This new method could provide reliable and useful data for environmentalists and agriculturalists to prevent MeHg pollution, facilitating the improvement of food safety for harvests from paddy soil.

1. Introduction

The amount of mercury in the top layer of the world’s oceans, down to 100 m, is now approximately twice as high as it was 100 years ago and is increasing (United Nations Environment Programme, UNEP 2013). Mercury in the atmosphere is emitted mainly from Asia, accounting for approximately 50% of global emissions (UNEP 2013). Mercury is transported over a long distance from the emission area to the seas around Japan through the atmosphere and ocean currents.

Since a part of inorganic mercury is converted to methylmercury (MeHg) by sulfate- and iron-reducing bacteria and methanogens under anaerobic conditions, such as paddy soils (Liu et al. 2018), the soils polluted by inorganic mercury cause problems in MeHg-polluted rice in China (Lin et al. 2021). Kodamatani et al. (Kodamatani et al. 2020) indicated that MeHg generated in paddy soil was transferred to rice grains during rice plant growth, with the highest Hg concentration in the embryo. Furthermore, Du et al. (Du et al. 2021) indicated that MeHg bioaccumulated in edible organisms, such as loaches and eels, living in mercury-polluted paddy fields. Although MeHg exposure through rice consumption as well as fish ingestion is of concern, particularly in Southeast Asia, detailed information is lacking (Rothenberg et al. 2014). In the future, if paddy soil is further polluted by inorganic mercury in Japan, more MeHg-polluted rice will be produced, and there will be an increased risk to health through long-term and chronic MeHg exposure from staple rice.

Therefore, a universal method for measuring the concentration of MeHg in soils is required to prevent pollution. The Japanese official mercury analytical method for soils (The Ministry of the Environment 2004) uses harmful reagents that are difficult to handle, such as dithizone toluene solution for extraction, alkaline solution for decomposition, and toluene for pre-cleaning reagents. The method also requires a
device with restricted use, such as a gas chromatograph system connected with a radioactive $^{63}$Ni electron capture detector (GC-ECD).

A solid-phase cartridge for sample preparation is a more user-friendly tool for MeHg quantification than alkaline solutions and toluene-based techniques. Thus, MeHg in soils could be more efficiently quantified with high specificity and validation performance using a universal technique: gas chromatography-mass spectrometry (GC-MS). In this study, we aimed to develop a simple and universal method for quantifying MeHg in soils. Using this newly developed method, we assessed the MeHg levels in the reference samples.

2. Materials And Methods

2.1. Chemicals and reagents

Methylmercury chloride (98% purity) was purchased from GL Science Inc. (Tokyo, Japan). Potassium bromide, sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate (guaranteed reagent grade, respectively), anhydrous sodium sulfate, acetonitrile, acetone, and n-hexane (for pesticide residue-PCB analysis, respectively), polyethylene glycol 200 (PEG200), formic acid (Wako 1st grade, respectively), and hydrochloric acid (for analysis of poisonous metals) were purchased from FUJIFILM Wako Pure Chemical Corporation, Ltd. (Osaka, Japan). Tetraphenyl sodium borate (Kalibor, analytical grade) was purchased from Dojindo Laboratories Co., Ltd. (Kumamoto, Japan). The Oasis Prime HLB cartridge (200 mg/6 cc) and Sep-Pak C18 Vac 3 cc (200 mg) were purchased from Waters (Milford, MA, USA). Glass beads (cat. no. BZ-1) were purchased from As One Co., Ltd. (Tokyo, Japan) and washed with acetone and n-hexane before use. Potassium bromide solution (1 mol/L) was prepared by dissolving 59.5 g of potassium bromide in 500 mL of water. Hydrochloric acid (5 mol/L) solution was prepared by adding 150 mL of concentrated hydrochloric acid to 210 mL of water. A 0.2 mol/L sodium phosphate buffer (pH 7.0) was prepared by mixing 0.2 mol/L sodium dihydrogen phosphate solution with 0.2 mol/L disodium hydrogen phosphate solution and adjusting the pH. A 1% sodium tetraphenylborate solution was prepared using sodium phosphate buffer. Ultrapure water was used in all experiments. Glassware was washed with acetone and n-hexane before use.

2.2. Sample extraction

Certified estuarine sediment samples of ERM CC-580 containing 132 ± 3 mg/kg of mercury and 0.075 ± 0.004 mg/kg of MeHg were obtained from the Institute for Reference Materials and Measurements (Geel, Belgium). The Geological Survey of Japan (GSJ) geochemical reference samples of sediments in Japan: JLK-1 collected from Biwa Lake; JSD-1, 2, 3, and 4 collected from the Kanto area; and andosol samples—JSO-1—collected from Tokyo, were obtained from the National Institute of Advanced Industrial Science and Technology (Ibaraki, Japan). Environmental certified reference material of landfill cover soil—No. 33—collected from north of Tokyo in Japan was obtained from the National Institute for Environmental Studies (Ibaraki, Japan), which was irradiated at a high dose of 21 kGy with a cobalt source.
Methylmercury was extracted from each 1.0 g sample using 10 mL of 5 mol/L hydrochloric acid solution, 5 mL of 1 mol/L bromide potassium solution, and 4 g glass beads for 5 min with shaking at 2,500 rpm. After the samples had been centrifuged at 20°C and 3,589 × g for 5 min, the residue was extracted again as described above. The resulting supernatants were collected as extracts.

2.3. Preparation of test sample solutions

The extracts were loaded into an Oasis PRiME HLB solid-phase cartridge conditioned with 6 mL of acetonitrile and 6 mL of water before use. The cartridge was washed with 10 mL of water and eluted with 5 mL of acetonitrile containing 10% formic acid. The elute was adjusted with the same solution until a total volume of 5 mL, and 4 mL of elute was used for phenyl-derivatization by mixing with 5 mL of phosphate buffer, 1 mL of sodium tetraphenylborate solution, and 2 mL of n-hexane, followed by shaking at 2,500 rpm for 2 min. After centrifugation at 20°C and 3,589 × g for 3 min, the n-hexane phase was dehydrated by adding anhydrous sodium sulfate, and then, 0.2 mL of n-hexane phase was added to 0.1 mL of 1.5 mg/mL PEG 200. The resulting solution was used as the test sample for the subsequent GC-MS analysis.

Three micrograms per milliliter of MeHg solution, a spiking agent, was prepared by dissolving 11.64 mg of methylmercury chloride in water to 100 mL with ultrasound treatment and diluting 3 mL with water to 100 mL. A 1,000 mg/L MeHg stock standard solution was prepared by dissolving 11.68 mg of methylmercury chloride in 10 mL of toluene. The stock standard solution was diluted with n-hexane to cover a 1–50 ng/mL concentration range, providing a full calibration curve.

2.4. GC-MSD analysis

The test sample solution was assayed with a 5973 inert gas chromatograph equipped with a mass spectrometry detector (GC-MSD; Agilent Technologies, Inc., Santa Clara, CA, USA). Separation was carried out in an InertCap 5MS/NP (30 m × 0.25 mm i.d., 0.25 µm film thickness) column (GL Sciences Inc.) using helium as the carrier gas (flow rate: 1.0 mL/min). The injector temperature was 250°C, and the injection volume was 1 µL (splitless). The oven temperature was programmed from 70°C (1 min) to 280°C (5 min) at a rate of 20°C/min, and the transfer line temperature was set to 280°C. The mass spectrometer was operated in the electron ionization mode with selected ion monitoring (SIM). Selected ions were monitored at m/z 294 (quantitative ion) and 292 (confirmative ion), and the ion source temperature was set to 230°C. The concentrations obtained from the GC-MSD analysis were converted to units of nanograms per gram of sample weight by multiplying the corresponding dilution factor to obtain the MeHg levels.

2.5. Method validation

One gram of certified estuarine sediment sample (ERM CC-580) was extracted without spiking. One gram of GSJ geochemical reference samples (both JLK-1 and JSO-1) were spiked prior to extraction with MeHg at a concentration of 20 ng/g. The MeHg levels of spiked and non-spiked test sample solutions were measured twice per assay and once per assay, respectively, in six independent experiments in a single
laboratory, performed according to the official Japanese guideline of analytical methods for metals in foods (The Ministry of Health 2012). The average recovery and intra- and inter-assay reproducibility were calculated using a one-way analysis of variance (one-way ANOVA). As a result, a six-point calibration curve was generated. The concentration was calculated in the range with signal-to-noise (S/N) ratios of more than three in the qualitative analysis and more than ten in quantitative ion detection.

3. Results And Discussion

3.1. Optimization of analytical method and sample preparation

Solid-phase cartridges are simple, easy-to-use tools that can be extracted and purified without hazardous reagents; hence, they have been widely used for pesticide analyses (Amendola et al. 2010; Schenck et al. 2008). Liem-Nguyen et al. (2020) reported a MeHg analytical method using a solid-phase cartridge to concentrate water samples and improve the test sensitivity. However, the solid-phase cartridge is also potentially suitable for removing the soil matrix, thus facilitating the use of GC-MSD, which is susceptible to the negative effects of the soil matrix. After loading the Oasis PRiME HLB solid-phase cartridge with the extracts, the hydrophobic methyl group of MeHg bound to the HLB cartridge, and the aqueous matrix was removed by washing with water, followed by elution with acetonitrile containing 10% formic acid. To the best of our knowledge, this is the first study to develop a GC-MS-based method combining a solid-phase cartridge with high selectivity and validation performance.

To optimize sample preparation, we first tested the extraction volume with 1 mol/L of potassium bromide solution and 5 mol/L of hydrochloric acid solution. The former was determined to be 10 mL in total. The latter was determined to be 20 mL in total, as the recovery rates of MeHg in ERMCC-580 were best between 92.1%, 99.7%, and 105.2%, corresponding to 10, 20, and 30 mL of the total volume of 5 mol/L of hydrochloric acid solution, respectively (data not shown).

Next, to determine the optimal type of solid-phase cartridge, we tested the recovery rates between the Sep-Pak C18 Vac (200 mg, 3 cc) and Oasis PRiME HLB cartridges (200 mg, 6 cc) by adding 100 ng of MeHg spiking agent to the extracts from the GSJ geochemical reference sample of JSO-1 that did not contain MeHg. Both the Sep-Pak C18 Vac and Oasis PRiME HLB cartridges were capable of retaining MeHg with hydrophobic bonds. However, with the use of 10 mL of acetonitrile, MeHg was not recovered from the former, whereas approximately 46% of MeHg was recovered from the latter. We speculated that this was because the Sep-Pak C18 Vac cartridge had a higher Log P of 9.18 and silanol groups that absorbed basic compounds of MeHg with ion-exchanges, whereas the polymer-based Oasis PRiME HLB cartridge had a lower log P of 3.59 and did not have silanol groups. Since the recovery rate with the Oasis PRiME HLB cartridge was almost the same when the loading volume was between 60 mg, 200 mg, and 500 mg, 200 mg was selected to minimize the flow time and elution volume. To improve the MeHg elution efficiency with the Oasis PRiME HLB cartridge, acetonitrile containing formic acid, which enhanced the dissociation of the ionic bond between methylmercury and bromide and chloride ions, was tested as an
elution solvent. As a result, the recovery rates of JSO-1 extracts spiked with 100 ng of MeHg were 102% and 106%, eluting with 10 mL of 10% and 15% formic acid/acetonitrile, respectively. The 10% formic acid solution was used for elution to minimize the amount of formic acid.

According to a previous report (The Ministry of the Environment 2014), the average MeHg concentration of soil and sediment (n = 288) was 21.39 ng/g with a maximum value of 444.35 ng/g. To verify the applicability of the elution method to the MeHg concentration level of the real sample and the MeHg retention capacity of the solid-phase cartridge, JSO-1 extracts spiked with 20 ng and 600 ng of MeHg were tested. The MeHg was eluted with 2.5 mL of 10% formic acid/acetonitrile and fractioned four times. The recovery rates of MeHg are summarized in Table 1. Although approximately 100% of MeHg was recovered in the first fraction during testing of both 20 ng and 600 ng of MeHg spiked samples, small amounts (approximately 3 ng/mL, namely 8 ng) of MeHg were confirmed in the second fraction during testing of 600 ng of MeHg spiked samples. Therefore, the elution volume was set to 5 mL for full recovery. In this manner, the newly developed elution method had sufficient performance to cover MeHg levels in real sample analysis.

| Sample | Spiked MeHg concentration (ng/g) | Fraction volume (mL) | Recovery (%) † |
|--------|----------------------------------|----------------------|----------------|
| JSO-1  | 20                               |                      | 106.1          |
|        | 600                              | 2.5                  | 104.6          |
|        |                                  | 5                    | 1.3            |
|        |                                  | 7.5                  |                |
|        |                                  | 10                   |                |
| †: The values were less than limit of detection. |
| † Mean recovery (%) at n = 2. |

### 3.2. Method validation

In this method, extracted and purified MeHg was phenyl-derivatized and analyzed using GC-MS in the SIM mode, monitoring $m/z$ 294 ion (quantitative ion) and $m/z$ 292 ion (confirmative ion), respectively. Typical chromatograms of the phenyl derivative MeHg in the standard solution and test sample solutions are shown in Fig. 1. The approximate retention time of the MeHg was 5.5 min. Quantitative ion calibration curves of phenyl-derivatized MeHg were generated with excellent linearity ($r^2$ greater than 0.99) in the range of 1–50 ng/mL (Fig. 2). The limits of detection (LOD) and quantification (LOQ) were determined to be 1 ng/mL and 3 ng/mL (2.5 ng/g and 7.5 ng/g each converting to per sample weight), calculated at $S/N$ ratios greater than 3 and 10, respectively.
The validation parameters were determined in six independent experiments, including trueness and reproducibility, and are summarized in Table 2. The trueness values were good and were 98.8%, 102.6%, and 96.2% for ERMCC-580, JLK-1, and JSO-1 extracts, respectively. The repeatability and reproducibility were high: 3.4%, 7.1%, and 7.0%, and 4.3%, 7.1%, and 7.0%, respectively, as determined by the intra-assay coefficient of variations (CVs) and the inter-assay CVs for ERMCC-580, JLK-1, and JSO-1 extracts, respectively. The trueness and intra- and inter-assay CVs were considered acceptable, according to the Japanese official guidelines for the validation of test methods for metals (The Ministry of Health, Labour and Welfare of Japan 2012).

| Sample      | Spiked MeHg concentration (ng/g) | Recovery (%) | Intra-assay CV (%) | Inter-assay CV (%) |
|-------------|---------------------------------|--------------|--------------------|--------------------|
| ERMCC580 a  | –                               | 98.8         | 3.4                | 4.3                |
| JLK-1       | 20                              | 102.6        | 7.1                | 7.1                |
| JSO-1       | 20                              | 96.2         | 7.0                | 7.0                |

a Certified estuarine sediment sample containing 0.075 ± 0.004 mg/kg of MeHg.

b Mean recovery (%) in six independent assays.

Since a high level of inorganic mercury is distributed biasedly in soil and sediment samples, there is a risk of overestimating MeHg concentration values unless a reliable separation of methylmercury from inorganic mercury is achieved. (Morita et al. 1982). As mentioned above, we demonstrated the high validation performance of the GC-MSD method in samples despite certain difficulties. Specifically, the difficulties were that ERMCC-580 had a significantly biased methyl and inorganic mercury content ratio (approximately 1760-fold difference), and JSO-1 of andosol soil had a complex matrix, including humic substances, causing many analytical problems (Łozowicka et al. 2017). Therefore, this newly developed solid-phase extraction-based method is a clean and simple method that can be used with a universal GC-MSD device, which reduces the use of harmful reagents, overcoming challenges encountered in previous studies.

### 3.3. Methylmercury concentration measurements

The newly developed method was used to assess MeHg levels in GSJ geochemical reference samples JSD-1–4 and the environmental certified reference material No. 33, and the results are summarized in Table 3. The MeHg concentration of JSD-4 was determined to be 64.1 ng/g, whereas MeHg was not detected in other samples of JSD-1–3 and No. 33 (less than LOQ). However, these MeHg-negative samples contained certified concentration values of total mercury as follows: 16, 106, 254, and 89 ng/g,
respectively. For JSD-4, there was no information on the certified concentration of total mercury. Overall, the resulting values of MeHg in Table 3 were acceptable because they were well below 15 mg/kg, the maximum limit set by Japanese law for the regulation of mercury and its compounds to prevent soil pollution.

Table 3
Total mercury and MeHg levels in five types of reference samples.

| Sample          | Concentration (ng/g) | MeHg  |
|-----------------|----------------------|-------|
|                 | Total mercury b      | MeHg c|
| JSD-1           | 16                   | N.D.  |
| JSD-2           | 106                  | N.D.  |
| JSD-3           | 254                  | N.D.  |
| JSD-4           | –                    | 64.1  |
| No. 33          | 89                   | N.D.  |
| N.D.: < 7.5 ng/g. |                     |       |
| −: No information on certified concentration value. | | |

a Samples were collected from Japanese soil and sediment.
b Certified values determined by manufacturer.
c Each value was determined in n = 1.

As some inorganic mercury is converted to MeHg by anaerobic bacteria remaining in soil and sediment samples during storage (Kodamatani et al. 2017), the MeHg levels can vary. Therefore, the collected samples were stored frozen (Kodamatani et al. 2017). As shown in Table 3, with the exception of No. 33, which was irradiated at a high dose for sterilization, the MeHg concentrations of JSD-1–4 may be variable in other assays because JSD-1–4 was not verified as completely sterilized by the manufacturer. Nonetheless, the concentration values of JLK-1 and JSO-1, which were not verified for complete sterilization and obtained from the same manufacturer as JSD-1–4, were considered to be constant in method validation; therefore, the JSD-1–4 concentrations could also be constant, and the range of variation was considered to be small. Overall, this method can be applied to the surveillance of MeHg pollution in soils, but future studies will require optimal soil sterilization treatment and verification of concentration variance to obtain more accurate concentration values.

In conclusion, we developed a solid-phase extraction-based method for quantitative methylmercury analysis of soil and sediment samples. This is a clean and simple method using a universal GC-MSD device, which reduces the use of harmful reagents and demonstrates excellent trueness, repeatability, and
reproducibility. The MeHg levels of the reference soil and sediment samples were measured, and the values were acceptable for the Japanese regulatory criteria. This newly developed method can provide reliable and useful data and help environmentalists and agriculturalists prevent MeHg pollution. It may also facilitate food safety improvements for rice grain and edible organisms harvested from paddy soil.

Declarations

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Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

Sachiko Kakimoto: Conceptualization, Resources, Methodology, Formal analysis, Validation, Investigation, Writing – original draft, Funding acquisition.

Masato Yoshimitsu: Conceptualization, Methodology, Validation, Writing - review & editing.

Kyohei Kiyota: Visualization, Writing - review & editing.

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Figures

Figure 1
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Figure 1

Extracted quantitative ion (m/z 294) chromatogram obtained using GC-MSD analysis (a) JLK-1 and (b) JSO-1 extracts spiked with 20 ng/g of MeHg. (c) 3 ng/mL standard MeHg solution. (d) JLK-1 and (e) JSO-1 extracts spiked with blank solution.
Figure 2

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Figure 2

Typical six-point calibration curves of the phenyl derivative MeHg for quantification at m/z 294, having a range of 1–50 ng/mL.