Application of enriched stable $^{196}$Hg isotope for monitoring the stability of total mercury in water samples

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A novel tool for the investigation of stability of total mercury in water samples is presented. The study focuses on the application of enriched $^{196}$Hg stable isotopic reagent for the stability studies. Natural abundance of $^{196}$Hg in water samples is only 0.15%. Thus, the use of the $^{196}$Hg isotope spike represents a major advantage, when it can be assumed that all the measured isotope is the same as is accurately added by the analyst, and the change in its mass concentration can be followed simply and reliably. Tests were carried out with industrial waste water and two type of the natural water. Cold vapour (CV) inductively coupled plasma mass spectrometer (ICP-MS) technique was applied for the mercury measurements. Monitoring was continued for approximately 100 days. It is commonly advised that the measurement for total mercury in water samples should be carried out within 14 days. In this study the samples were observed to be stable for more than three months, if they were stored at a temperature of 4–6°C. The results of this stability study were in line with the guidance presented in EPA standard 1631. However, the samples were noticed to be stable for a much longer time than is presented in the standard method ISO 17852.

**Keywords:** total mercury; stability study; storage time; enriched isotope; water sample

1. Introduction

Mercury is a metal that is harmful or toxic in the water environment. The common aqueous species of mercury are $\text{Hg}^{2+}$, $\text{Hg(OH)}_2^-$, $\text{Hg}^0$, and stable complexes with organic ligands [1]. These may form mono- or di-substituted organometallic compounds through covalent bonding to phenyl or short-chained alkyl substituents, yielding for example toxic dimethylmercury ((CH$_3$)$_2$Hg), and methylmercury (CH$_3$Hg$^+$) [2].

The EU Member States are obliged to monitor the mass concentration of total mercury and its compounds in the water environment according to the Water Framework Directive (2000/60/EC) [3] and in particular the Priority Substances Directive [4]. These directives set the environmental quality standards (EQS) for the substances in e.g. surface waters (river, lake, transitional and coastal) and confirm their designation as priority or priority hazardous substances. In the Priority Substances Directive, the total concentration of mercury (and other metals) refers to the total dissolved concentration, i.e. the dissolved phase of a water sample obtained by filtration through a 0.45 μm filter [4].

For routine testing laboratories, there are several guides and standards available for the measurement of total mercury in water samples. The mass concentration of mercury in natural

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water is usually very low (ng L$^{-1}$) and a wide variety of natural water matrices exist. There are different recommendations available for storage times of water samples intended for total mercury measurement. Two most essential standard methods are the ISO 17852 [5] and EPA Method 1631 [6]. The first one suggests that samples should preferably be preserved on site using hydrochloric acid, potassium bromide and potassium bromate reagents. ISO 17852 suggests also carrying out total mercury measurement within seven days of collection. According to EPA Method 1631 [6], the samples for total mercury measurement should be either preserved or measured within 48 hours of collection. EPA 1631 suggests preserving samples with BrCl reagent containing hydrochloric acid, potassium bromide and potassium bromate. After preservation the samples are stable for up to 90 days from the date of collection [6].

From other standards available the ISO 12846 [7] suggests measuring total mercury within two weeks of sampling. Standard methods for the examination of water and waste water [8] give 28 days as the maximum storage time, whereas ASTM standard D3223-12 [9] gives 38 days. Standard ISO 5667-3 [10] presents the maximum storage time to be as long as six months using preservation with HNO$_3$ to pH in the range of 1 to 2.

Variations in the Hg concentration over time in the water samples may be occurring due to several causes. These include sample characteristics, Hg speciation, sample pre-treatment, the chemical conditions of preservation, the material of the sample container, storage conditions, etc. Leermakers et al. [11] presented two mechanisms, which are responsible for the loss of mercury from solution. These are volatilisation of the analyte and its adsorption on the container surface. Hg$^{2+}$ can be reduced to Hg$^{1+}$ in the presence of a reducing agent occurring either naturally (i.e. micro-organisms, humic acids) or as an impurity in solution. Hg$^{1+}$ will then disproportionate spontaneously producing volatile mercury species, which escape from the solution. Low pH and high ionic strength prevents adsorption on the container walls, while oxidising and complexing agents keep the inorganic mercury as Hg$^{2+}$ [11]. According to present knowledge, the best approach for preserving water sample for total Hg measurement is to add BrCl reagent containing hydrochloric acid, potassium bromide and potassium bromate directly in the sample bottle at least 24 hours before measurement [6]. EPA Method 1669 states that preserved samples should be allowed to stand for at least two days to allow the metals adsorbed to the container walls to redissolve [12].

Many problems are also encountered with polyethylene (PE) bottles. Active sites on the interior wall surface (such as hydrocarbon radicals and carbonyl groups) and additives (amino, thiol, sulphide of phenolic groups) can cause mercury loss by adsorption and reduction. Adsorbed mercury is also very hard to remove from contaminated surfaces [11]. Hammerschmidt et al. [13] have recently studied storage bottle materials and cleaning procedures for measurement of trace-level total mercury in seawater. They noticed that Hg in seawater stored in low-density polyethylene (LDPE), fluorinated high-density polyethylene (FLPE), and fluorinated ethylene propylene (FEP) bottles increased within 15 weeks of storage at room temperature. In contrast, Hg levels in seawater stored in polycarbonate or glass bottles were increased modestly after 74 weeks or they remained even unchanged throughout the test [13]. Different approaches for applying enriched mercury isotopes for investigations of mercury behaviour in the environment have been presented. One of the recent studies have been presented by Ribeiro Guevara and Horvat [14] by studying the stability and partitioning of inorganic mercury (Hg$^{2+}$) in natural waters under different storage conditions. The experiments were carried out by spiking labelled Hg$^{2+}$ to natural concentrations (from 3 to 13 ng L$^{-1}$) in marine, coastal lagoon, lake, river, and rain waters. Hg$^{2+}$ was labelled with high specific activity $^{197}$Hg. The tests were carried out 10–13 days, and the concentration of labelled Hg$^{2+}$ in non-preserved waters decreased significantly depending on
the type of water and the storage conditions. In the case of marine and lake waters it was stable over a period of 10 days, whereas in the case of river and lagoon waters 20% of labelled Hg was lost. The labelled Hg$^{2+}$ in water samples preserved by 1% HCl addition was stable at room temperature (20°C) and in a refrigerator (5°C) when stored in Teflon and borosilicate glass, but significant losses were observed when stored in polyethylene [14]. Ribeiro Guevara et al. [15] and Žižek et al. [16] have also presented an application using $^{197}$Hg radiotracers for the study of mercury transformations in environmental systems, particularly mercury methylation, demethylation, and reduction in sediments and water. Hintelmann et al. [17] used stable mercury isotopes to investigate the possibility of CH$_3$Hg$^+$ generation during sample preparation, in particular from sediments.

However, this study was focused on total mercury measurements only. In the determination of total mercury, samples are preserved with BrCl reagent containing hydrochloric acid, potassium bromide and potassium bromate. The BrCl reagent oxidises Hg compounds to Hg$^{2+}$. This includes Hg$^{2+}$, Hg$^0$, strongly organo-complexed Hg$^{2+}$ compounds, absorbed particulate Hg, and several covalently bound organo-mercurials (e.g. CH$_3$HgCl, (CH$_3$)$_2$Hg and C$_2$H$_5$HgOOCCH$_3$) [6].

The aim of this study was to develop a novel tool for the investigation of the stability of total mercury in water samples and apply it for monitoring the stability of two different proficiency test (PT) samples and one natural water sample during the storage. The tool presented is the application of an enriched $^{196}$Hg stable isotopic reagent in stability studies. The natural abundance of $^{196}$Hg is 0.015% in water samples. By adding an accurately known amount of enriched $^{196}$Hg isotope to the samples, reliable information on the alteration of the mercury concentration can be obtained.

2. Experimental

2.1 Reagents and standard solutions

Reagents and standard solutions with appropriate purity for avoiding mercury contamination should be used.

Ultrapure water (resistivity 18 MΩ cm$^{-1}$), prepared with Millipore Elix® 70 and followed by purification with the Millipore A10® water purification system including 0.1 µm filtering step, was used throughout.

All sampling vessels and reagent solution containers were soaked in (1 + 1) nitric acid overnight and rinsed several times with ultrapure water prior to use. The autosampler polystyrene test tubes were found to be clean and were not rinsed before use.

Hydrochloric acid (super purity acid; Romil-SpA®, Cambridge, UK) was used in samples. The final concentration of the HCl used was 0.6 mol L$^{-1}$ (5%; V/V).

Tin(II)chloride (2%; m/V) was prepared daily by dissolving the appropriate amount of SnCl$_2$·2 H$_2$O (Merck, Darmstadt, Germany) in ultrapure water. The prepared solution was sparged with argon for 15 minutes prior to use.

The ascorbic acid solution (1%; m/V) was prepared by dissolving 10 g of C$_6$H$_8$O$_6$ (Merck) in 100 mL of ultrapure water. The solution was prepared weekly.

The potassium bromide stock solution was prepared by dissolving 4.76 g of KBr (Merck) in 200 mL of ultrapure water. The solution was prepared weekly.

The potassium bromate stock solution (0.6%; m/V) was prepared by dissolving 1.12 g of KBrO$_3$ (Merck) in 200 mL of ultrapure water. The solution was prepared weekly.

The KBr + KBrO$_3$ solution was prepared fresh before use by mixing equal amounts of KBr and KBrO$_3$ stock solutions.
Balances were used for the preparation of stock solution and diluted working solutions of the $^{196}$Hg. Weighing of the stock solution was carried out using a calibrated analytical balance (Mettler-Toledo XP56) with a resolution of 1 µg. Weighing of the working solutions was carried out using a calibrated analytical balance (Radwag PS 1000.3Y) with a resolution of 1 mg.

The stock solution of $^{196}$Hg (9.767 mg kg$^{-1}$) was prepared by dissolving 5.35 mg of enriched HgO (Trace Sciences International Inc., Richmond Hill, Canada) in approximately 10 mL of nitric acid (1:1) in a quartz test tube. The solution was quantitatively transferred into a 500 mL bottle with 5% (V/V) HCl. The abundance of the $^{196}$Hg was 52.00 ± 0.20% in the enriched HgO. Isotopic composition of the product is presented in Table 1. The chemical purity of the material was 99.9%.

Working Solution 1 (spike solution for waste water) of $^{196}$Hg (61.31 µg kg$^{-1}$) was prepared by diluting 3.047 g of $^{196}$Hg stock solution in approximately 500 mL of 5% (V/V) HCl.

Working Solution 2 (spike solution for high concentration natural water) of $^{196}$Hg (3.69 µg kg$^{-1}$) was prepared by diluting 15.028 g of $^{196}$Hg Working Solution 1 in approximately 250 mL of 5% (V/V) HCl.

Working Solution 3 (spike solution for low concentration natural water) of $^{196}$Hg (0.496 µg kg$^{-1}$) was prepared by diluting 4.045 g of $^{196}$Hg Working Solution 1 in approximately 500 mL of 5% (V/V) HCl.

The standard solutions for the external calibration curve were prepared daily by diluting the appropriate amount of $^{196}$Hg stock and working solutions. To monitor the evaporation, the $^{196}$Hg stock and working solutions were weighed before and after each time they were used.

Argon gas with a purity of 99.999% was used throughout this study.

### 2.2 Sample types tested

Three different sample types were tested. Two of these were PT samples and the aim was to find out how long the SYKE Proftest (PT provider of Finnish Environment Institute, Finland) can store the PT samples after their preparation. The PT samples were collected several months before homogenisation and sample division. The PT sample matrices were natural river water (sample code N1) and industrial waste water (sample code IW).

The natural river water (N1) with a high total Hg mass concentration (ca. 150 ng L$^{-1}$) was prepared by collecting natural water in autumn 2011 and allowing it to stand for 20 months at a temperature of $+4^\circ$C. After that the suspended solids, which settled at the bottom of the container, were separated from the rest of the water by decanting. The water sample was preserved with hydrochloric acid (50 mL conc. HCl per 1 litre of sample water) and spiked with mercury standard solution with natural isotopic composition before dividing it into smaller bottles. The characteristic (matrix) of the sample N1 (see Supplemental online material) is quite similar than the matrix of the sample N2, but the mercury mass concentration is higher.

The industrial (treated) waste water (IW) was collected in spring 2012 from a factory producing stainless steel. The waste water was preserved immediately after sampling with hydrochloric acid (50 mL conc. HCl per one litre of sample water) and after that it was allowed to stand for 12 months at room temperature. After that the suspended solids, which had settled

| Isotope | 196 | 198 | 199 | 200 | 201 | 202 | 204 |
|--------|-----|-----|-----|-----|-----|-----|-----|
| Enrichment (%) | 52.00 ± 0.20 | 5.00 ± 0.05 | 31.15 ± 0.10 | 3.20 ± 0.05 | 4.20 ± 0.05 | 3.50 ± 0.05 | 0.95 ± 0.03 |
to the bottom of the container, were separated from the rest of the water. Decanted water was divided into smaller bottles and the total Hg mass concentration of the samples was ca. 2000 ng L$^{-1}$. The characteristic (matrix) of the sample IW consisted following metals with mass concentration of higher than 100 µg L$^{-1}$: Al, Cr, Fe, Mn, Ni, and Zn.

In addition to the PT samples, natural water with a low total Hg concentration (ca. 10 ng L$^{-1}$, sample code N2) was tested. The sample was collected on 16 November 2013 from the Vanjoki, a river in southern Finland, ca. 20 h before starting the stability studies. Ten litres of sample was collected and it was acidified immediately after sampling with 500 mL of concentrated hydrochloric acid. Typical matrix parameters of this river water are presented as Supplemental online material.

### 2.3 Stability study protocol

Sample preparation steps for stability studies were carried out gravimetrically. The samples were prepared and tested in the following way:

1. 4–10 litres of each tested sample matrices (N1, N2 and IW) was collected.
2. Approximately 240 g of each sample type was divided homogenously using a magnetic stirrer into 250 mL borosilicate glass bottles, resulting in 17 bottles per matrix.
3. 13 of the samples in each matrix were spiked accurately with enriched $^{196}$Hg isotope (enrichment of $^{196}$Hg 52%, $n_{\text{ADD}}$($^{196}$Hg), $t_0$). The additions were:
   - (a) ca. 10.0 g of Working Solution 2 to the high concentration Hg sample (N1),
   - (b) ca. 5.0 g of Working Solution 3 to the low concentration Hg sample (N2),
   - (c) ca. 10.0 g of Working Solution 1 to the industrial waste water sample (IW).
4. Four of the samples in each matrix were not spiked.
5. Seven of the samples in each sample matrix were stored in the dark and at room temperature. The remaining six samples of each type were stored in the dark and at 4–6°C.
6. Total mercury mass concentration in each sample bottle was measured in quadruplicate in random order according to Table 2. Total mercury mass concentration in non-spiked samples was measured in the beginning of the study from four different borosilicate glass bottles, four measurements per bottle.

| Number of days | Sample code | 0 | 1 | 3 | 4 | 8 | 10 | 19 | 24 | 33 | 42 | 56 | 91 | 114 |
|---------------|-------------|---|---|---|---|---|----|----|----|----|----|----|----|----|
| N1            | NS/1–4 S$_{RT}$/6 | S/4 | S$_{RT}$/2 | S/1 | S$_{RT}$/4 | S$_{RT}$/1 | S/2 | S/3 | S/6 |
|               | NS/1–4 S$_{RT}$/4* | S$_{RT}$/6 | S$_{RT}$/3 | S/5 | S$_{RT}$/7 | S$_{RT}$/2 | S/7 | S$_{RT}$/5 |
| N2            | NS/1–4 S$_{RT}$/4* | S/3* | S/1 | S$_{RT}$/4* | S$_{RT}$/7* | S/1* | S/4* | S/2* | S/5* |
|               | NS/1–4 S$_{RT}$/3* | S$_{RT}$/6* | S/1 | S$_{RT}$/7* | S$_{RT}$/2* | S$_{RT}$/1* | S$_{RT}$/5* | S$_{RT}$/6* |
| IW            | NS/1–4 S$_{RT}$/3* | S/3 | S/6* | S/1* | S/4* | S/2* | S/5* |

Notes: The number of days describes the time spent from the bottling of the samples to the start of the CV-ICP-MS measurement.

*Typically the sample pre-treatment step (oxidation with BrCl reagent) was started within 24 h prior to the measurement stage.

NS = non-spiked sample
S = spiked sample (stored at 4–6°C temperature)
S$_{RT}$ = spiked sample (stored at room temperature)
The number after the spiked/non-spiked code is the order of bottling during sample preparation.
2.4 Instrumentation

Since the isotope measurement of mercury is carried out, mass spectrometric technique is required for application. A Perkin-Elmer ELAN® DRC II inductively coupled plasma mass spectrometer (ICP-MS) equipped with Elemental Scientific (ESI) MP² hydrideICP Hydride Generation System and SC-2 DX autosampler, controlled by ELAN software version 3.4, was used for the measurements. The cold vapour (CV) unit consists of a 4-channel peristaltic pump and a gas/liquid separator. The CV unit was connected directly to the neck of the cyclonic spray chamber, made of quartz and cooled by ESI Peltier cooling system (PC³).

The instrumental parameters are shown in Supplemental online material. They were mainly chosen according to the results of earlier validation studies and manufacturer’s recommendations. The sample introduction system is described also in Supplemental online material.

2.5 Measurement of ‘reference’ value

The stability was monitored using isotope ¹⁹⁶Hg. For most natural waters background amount content of ¹⁹⁶Hg could be basically neglected, since it’s nearly zero and nearly all measured ¹⁹⁶Hg during stability tests is resulting from the spike added. However in this section is presented how the background amount content (even near to zero) of ¹⁹⁶Hg is taken into account. This is reasonable especially for samples having higher mercury mass concentrations such as waste waters.

The total amount content of ¹⁹⁶Hg was calculated from the known amount content of spiked ¹⁹⁶Hg, and from experimentally measured mass concentration (which was converted to the amount content value) of ¹⁹⁶Hg in the original non-spiked sample (natural abundance 0.015%). This total amount content of ¹⁹⁶Hg at time ‘0’ (t₀) was converted back to mass concentration representing the ‘reference’ value for the other mass concentration values obtained later during the storage period.

The amount content of ¹⁹⁶Hg in the non-spiked sample at the beginning of the stability study (t₀; n₀(¹⁹⁶Hg)) was unknown and had to be solved. As can be seen from Figure 1, at t₀, the added (weighed) amount of ¹⁹⁶Hg was known. At time ‘1’ (t₁) mass concentration of ¹⁹⁶Hg was measured by CV-ICP-MS in the spiked sample containing the sum of original ¹⁹⁶Hg and the added ¹⁹⁶Hg. As can be seen from Figure 1, at t₁, also the mass concentration of ²⁰²Hg was measured in the non-spiked sample. In Equation (1) ²⁰²Hg mass concentration measurement result is calculated into absolute number of moles (amount content):

\[
n_{OR1}(²⁰²Hg) = \frac{c_{OR1}(²⁰²Hg) \cdot V_{OR1}}{M(²⁰²Hg)}
\]

Figure 1. Solving the unknown amount content of ¹⁹⁶Hg in the non-spiked sample at the beginning of the stability study (n₀(¹⁹⁶Hg); t₀).
where \( n_{\text{OR1}}(^{202}\text{Hg}) \) is the amount content (in moles) of the Hg isotope 202 in the original non-spiked sample at the \( t_1 \),

\( c_{\text{OR1}}(^{202}\text{Hg}) \) is the measured mass concentration (in kg L\(^{-1}\)) of the Hg isotope 202 in the original non-spiked sample at the \( t_1 \),

\( V_{\text{OR1}} \) is the volume (in L) of the original non-spiked sample, and

\( M(^{202}\text{Hg}) \) is the molar mass (in kg mol\(^{-1}\)) of the Hg isotope 202.

Because it is difficult to measure accurately mass concentration (and therefore the number of atoms) of \(^{196}\text{Hg} \) in the sample containing \(^{199}\text{Hg} \) at background level, this was calculated indirectly by applying measurement result of more abundant \(^{202}\text{Hg} \) isotope in non-spiked sample \((n_{\text{OR1}}(^{202}\text{Hg}))\) at \( t_1 \) and using tabular values of natural abundances of mercury isotopes [18] as presented in Equation (2):

\[
n_{\text{OR1}}(^{196}\text{Hg}) = \frac{A(^{196}\text{Hg})}{A(^{202}\text{Hg})} \cdot n_{\text{OR1}}(^{202}\text{Hg}) \tag{2}
\]

where

\( n_{\text{OR1}}(^{196}\text{Hg}) \) is the amount content of original sample \(^{196}\text{Hg} \) at \( t_1 \), and

\( A(^{196}\text{Hg}) \) and \( A(^{202}\text{Hg}) \) are natural abundances of Hg isotopes 196 and 202.

By assuming that the possible change in the amount content of \(^{196}\text{Hg} \) during the storage affects both added \(^{196}\text{Hg} \) and original \(^{196}\text{Hg} \) isotope similarly between times \( t_0 \) and \( t_1 \) (Figure 1), it can be concluded that:

\[
\frac{n_{\text{AD0}}(^{196}\text{Hg})}{n_{\text{AD1}}(^{196}\text{Hg})} = \frac{n_{\text{OR0}}(^{196}\text{Hg})}{n_{\text{OR1}}(^{196}\text{Hg})} \tag{3}
\]

where

\( n_{\text{AD0}}(^{196}\text{Hg}) \) is the amount content of added \(^{196}\text{Hg} \) at the time of sample preparation \((t_0)\),

\( n_{\text{AD1}}(^{196}\text{Hg}) \) is the amount content of added \(^{196}\text{Hg} \) at \( t_1 \), and

\( n_{\text{OR0}}(^{196}\text{Hg}) \) is the amount content of original sample \(^{196}\text{Hg} \) at the time of sample preparation \((t_0)\).

Thus, the amount content of the isotope \(^{196}\text{Hg} \) in the original non-spiked sample at the \( t_0 \) can be resolved from Equation (3) and is calculated as follows in Equation (4):

\[
n_{\text{OR0}}(^{196}\text{Hg}) = \frac{n_{\text{AD0}}(^{196}\text{Hg}) \cdot n_{\text{OR1}}(^{196}\text{Hg})}{n_{\text{AD1}}(^{196}\text{Hg})} \tag{4}
\]

The amount content of the isotope \(^{196}\text{Hg} \) in the spiked sample at the \( t_1 \) is presented in Equation (5):

\[
n_{\text{AD1}}(^{196}\text{Hg}) = n_{\text{AD1}+\text{OR1}}(^{196}\text{Hg}) - n_{\text{OR1}}(^{196}\text{Hg}) \tag{5}
\]

When Equations (4) and (5) are combined, the following equation is obtained:

\[
n_{\text{OR0}}(^{196}\text{Hg}) = \frac{n_{\text{AD0}}(^{196}\text{Hg}) \cdot n_{\text{OR1}}(^{196}\text{Hg})}{n_{\text{AD1}+\text{OR1}}(^{196}\text{Hg}) - n_{\text{OR1}}(^{196}\text{Hg})} \tag{6}
\]
where

\[ n_{\text{AD1-OR1}}(^{196}\text{Hg}) \] is the amount content of \(^{196}\text{Hg}\) containing both original non-spiked \(^{196}\text{Hg}\) and added \(^{196}\text{Hg}\) measured at \( t_1 \), and it can be resolved from measured \(^{196}\text{Hg}\) mass concentration with the same principle as was shown for \( n_{\text{OR1}}(^{202}\text{Hg}) \) in Equation (1).

The total amount content of \(^{196}\text{Hg}\) in the spiked sample at \( t_0 \) \( (n_{X,0}) \) is then

\[ n_{X,0}(^{196}\text{Hg}) = n_{\text{OR0}}(^{196}\text{Hg}) + n_{\text{AD0}}(^{196}\text{Hg}) \]  

Obtained calculation result for \( n_{x,0} \) in moles can be turned back to mass concentration statement as follows:

\[ c_{x,0}(^{196}\text{Hg}) = \frac{n_{x,0}(^{196}\text{Hg}) \cdot M(^{196}\text{Hg})}{V_{x,0}(^{196}\text{Hg})} \]  

where

\( c_{x,0}(^{196}\text{Hg}) \) is the mass concentration (in g L\(^{-1}\)) of the Hg isotope 196 in the spiked sample at the \( t_0 \),

\( M(^{196}\text{Hg}) \) is the molar mass (in g mol\(^{-1}\)) of the Hg isotope 196, and

\( V_{x,0} \) is the volume (in L) of the spiked sample.

Since each sample was spiked with a slightly different amount of enriched \(^{196}\text{Hg}\) and the weighed mass of the sample was not exactly the same in each sample bottle, all the measurement results were normalised according to the theoretically calculated mass concentration of the \(^{196}\text{Hg}\) spike on the first day of measurement. Sample evaporation during storage was monitored and normalisation also included a factor for sample evaporation during the storage period (Equation (9)).

\[ c_{X,\text{Norm}} = c_X \cdot \frac{c_{0,\text{cal}}}{c_{X,\text{cal}} \cdot f_e} \]  

where

\( c_{X,\text{Norm}} \) is the normalised mass concentration of \(^{196}\text{Hg}\) at day \( X \),

\( c_X \) is the average of quadruplicate measurements for mass concentration of \(^{196}\text{Hg}\) at day \( X \), \( X > 0 \),

\( c_{0,\text{cal}} \) is the mass concentration of \(^{196}\text{Hg}\) spike added to the first sample measured at the beginning of the stability studies,

\( c_{X,\text{cal}} \) is the mass concentration of \(^{196}\text{Hg}\) spike added to the sample measured at day \( X \), \( X > 0 \),

\( f_e \) is the factor for evaporation correction.

The measurement result from Equation (8) is compared to the normalised measurement results obtained at different storage times.

### 2.6 CV ICP-MS measurement procedure

The CV ICP-MS instrumental set-up, parameters, standards and reagents used have been presented in Section 2 and in Supplemental online material. They were applied to the measurement of mercury in tested water samples. The measurement procedure with regards to sample pre-treatment (e.g. oxidation with BrCl) steps is based on the guidance written in the standard ISO 17852 [5]. It is designed for the measurement of total mercury in water by atomic fluorescence spectrometry, but is also appropriate for ICP-MS. For the ICP-MS measurement,
the standard procedures specifically for ICP-MS measurements were followed [19,20] taking into account the instrumental modifications required by application of CV technique shown in Supplemental online material.

Some 22–24 hours before the measurement, 10 mL of sample N2, 3 mL of sample N1 and 100 µL of sample IW were measured into the test tubes. Samples N1 and IW were diluted to 10 mL and 0.2 mL KBr+KBrO₃ mixture (1:1) was added to the samples to convert and oxidise inorganic and organic mercury species to Hg²⁺. This digestion procedure was allowed to continue overnight at room temperature. Immediately before measurement, 0.1 mL of ascorbic acid was added to the samples to remove excess bromine from the sample. The mass concentration of total mercury of the samples was measured according to the set-up and instrumental parameters described in Supplemental online material.

The stock solution of ¹⁹⁶Hg (9.767 mg kg⁻¹) was used for the preparation of the calibration standard (50 ng L⁻¹). The calibration standard was prepared fresh before each series of measurements. Two point calibration (0 and 50 ng L⁻¹) was used and the linear range was observed to continue to at least 200 ng L⁻¹. As there was no certified reference material available for ¹⁹⁶Hg, the synthetic control samples (5 and 50 ng L⁻¹) were prepared at each time of measurement by diluting working solutions 2 and 3.

Reagent blank samples were prepared for each sample run by adding 0.2 mL KBr + KBrO₃ mixture (1 + 1) into 10 mL of 5% (V/V) HCl. Immediately before measurement, 0.1 mL of ascorbic acid was added. Three to six blank samples were measured at the beginning of each run. The background signal of the mass spectrometer was monitored throughout the run by regularly measuring a rinse solution with a reagent matrix similar to the blank samples. Results of the control samples and reagent blank samples measured during stability study period are presented in the Supplemental online material.

2.7 Estimation of storage time

In the estimation of the stability of total mercury in water samples, the ASTM standard D4841 was consulted [21]. D4841 demonstrates the estimation of the ‘holding time’ (hereafter storage time) by means of replicate measurements at discrete time intervals using a large volume of a water sample that has been properly collected and preserved. Concentration of the constituent of interest is plotted over time. The maximum storage time is the period of time from sample collection to such a time that the degradation of a constituent of interest or change in the sample matrix occurs and the systematic error exceeds the confidence interval of the test calculated around the mean concentration [21].

The standard D4841 suggests calculating the range of tolerable variation (d; 99% confidence interval) according to Equation (10).

\[
d = \pm \frac{t \cdot s}{\sqrt{n}}
\]

\(d\) = Range of tolerable variation from the ‘reference value’ (ng L⁻¹)

\(t\) = Student's \(t\)-value for a two-tailed 99% confidence interval (\(t = 3.00\) was used)

\(s\) = Standard deviation of 10 parallel samples (ng L⁻¹)

\(n\) = Number of replicate measurements used at each time interval in the storage time determination.

In this study, the standard deviation in Equation (10) suggested by standard D4841 has been replaced by standard uncertainty (\(u_c\)) of the measurement result in the corresponding concentration range. This was chosen due to the fact that when measuring parallel samples during one
day, the typical day-to-day variation will not be included in the standard deviation. The standard uncertainty was calculated from the expanded measurement uncertainty by dividing it by the coverage factor \((k = 2)\). The expanded uncertainty \((U, k = 2)\) for total mercury measurement in natural water is 10% and for waste water 15% at the mass concentrations studied. The measurement uncertainties were estimated and calculated according to approach described in Nordtest handbook [22] and ISO standard 11352 [23] using information on internal quality control results for the estimation of within-laboratory reproducibility and the bias of the method and laboratory.

The tolerable range of variation (95% confidence interval) from the initial mean concentration that was used as the criterion for the storage time evaluation was calculated as follows:

\[
d = \pm k \cdot u_c
\]

where

- \(k\) = Coverage factor at 95% confidence interval \((k = 2\) was used),
- \(u_c\) = Standard uncertainty of the measurement (ng L\(^{-1}\)).

Division by the factor of \(\sqrt{n}\), as suggested in Equation (10), was not necessary, since \(u_c\) has been calculated according to approach described in Nordtest handbook [22] and ISO 11352 [23] taking into account the fact that replicate measurements were performed, and the standard deviation in estimation of systematic component is divided by \(\sqrt{n}\). This way the division by \(\sqrt{n}\) is included already in \(u_c\) itself. If \(u_c\) would be a significant part due to systematic effects, then dividing by \(\sqrt{n}\) would lead to an overly optimistic \(d\). In Figures 2–4, the changes in the mass concentration of \(^{196}\)Hg are graphically presented in samples N1, N2, and IW during storage times of up to 114 days. The tolerable variation is described with a dashed line around the ‘reference value’ of the \(^{196}\)Hg mass concentration. For industrial waste water (IW), the total mass concentration of \(^{196}\)Hg at \(t_0\) representing the ‘reference value’ could not be calculated reliably, and the value at \(t_1\) was used instead. The reason for this was the challenging matrix of the sample resulting in an incomplete oxidation of the waste water samples. Normally 0.5–1 h of oxidation time is required for water samples, but sample IW was not fully oxidised even after 24 h, and ca. 15% lower values were observed for \(^{196}\)Hg compared to the theoretical \(^{196}\)Hg mass concentration added to the samples. The effect of the oxidation time for the measurement result

Figure 2. Storage time determination for natural water N1 with two different storage temperatures. The initial \(^{196}\)Hg mass concentration was \((152 \pm 2)\) ng L\(^{-1}\). Dashed lines represent the range of tolerable variation. Each plot describes the average of four replicates with their standard deviation.
was already observed at the beginning of the stability studies, so in order to achieve comparable results, the oxidation time was kept as constant as possible for all studied IW samples.

3. Results and discussion

For investigated natural water (N1, Figure 2) with a high total mercury mass concentration (ca. 150 ng L\(^{-1}\)), the samples were found to be stable throughout the experimental period (114 days), when they were stored at a temperature of 4–6°C. A change in mass concentration was observed in samples stored at room temperature. The measured mass concentration exceeded the threshold value after a storage time of approximately 50 days.

For investigated natural water (N2, Figure 3) with a low mercury mass concentration (ca. 10 ng L\(^{-1}\)), the samples were regarded as stable throughout the experimental period (91 days), when they were stored at a temperature of 4–6°C. If they were stored at room temperature, the measured mass concentration exceeded the range of tolerable variation just at the end of the stability study (91 days).
According to this study, the stability time of investigated industrial waste water (IW, Figure 4) seems to be at least three months for both of the studied storage methods.

The reason for the observed decrease in the Hg mass concentration during the time was not further investigated, but the reason could be absorption of ionic species to the sample container walls or diffusion of dissolved Hg° from the sample through borosilicate glass material. Also Ribeiro Guevara and Horvat [14] noticed that the Hg concentration was decreased during the storage, but only for non-preserved samples. However in our study the changes were observed only after 7 weeks, while the study of Ribeiro Guevara and Horvat took less than two weeks [14]. Unlike us, Hammerschmidt et al. [13] noticed mercury concentration to increase during the storage. This was particularly observed using FEP, LDPE, and FLPE bottles. They suspected that the sample water was contaminated by diffusion of Hg° through bottle material. However it should be noticed that different natural and waste waters might behave differently. According to the nature of the water, mercury might be less stable than observed in this study.

The preparation of the samples for this stability study was rather easy, but careful planning in advance was required. The natural water sample (N2) was spiked about 20 h after sampling, so there is no exact information on how much the Hg mass concentration had changed between the time of sampling and the time of isotope addition. The sample was preserved with hydrochloric acid in the field at the time of sampling, which decreases the probability of the total concentration changing between the times of sampling and isotope addition.

The natural abundance of $^{196}$Hg isotope is only 0.15%. As a result, the background signal of the $^{196}$Hg isotope is very low in typical natural waters, where the total Hg mass concentration may be 1–20 ng L$^{-1}$. The background signal can be ignored, provided a sufficiently high spike of enriched $^{196}$Hg is used. This is an advantage since it can be assumed that all the measured mercury derives from the spike added by the analyst, and therefore any changes in mass concentration are easily monitored and assessed. If the $^{202}$Hg isotope (or any other common mercury isotope) would be used as a spike, then the problem would always be the accurate assessment of the ‘reference value’, since the original $^{202}$Hg mass concentration could not be ignored. Due to the low background, the use of enriched $^{196}$Hg instead of a more abundant natural isotope enables a much lower mass concentration of the spike, which is within the mercury mass concentration range present in natural waters.

In this study we assumed that the possible change in the mass concentration of $^{196}$Hg during the storage affects the natural Hg isotopes same way. There is a risk that the behaviour of spiked $^{196}$Hg is not exactly similar as natural Hg isotopes. The different behaviour is certainly true samples containing organic mercury species. The water samples investigated in this study were assumed to contain negligible mass concentrations of organo-mercury species. The water samples investigated in this study were assumed to contain negligible mass concentrations of organo-mercury species.

On one hand, it is important to keep the mass concentration $^{196}$Hg isotope higher than limit of the quantification (LOQ) of the measurement method, because otherwise the accuracy of the measurement results is reduced and the true changes in the Hg mass concentration will not be observed due to high random variation. The LOQ of 1 ng L$^{-1}$ for the measurement of total mercury in natural water by CV ICP-MS applied in this study was found to be suitable. On the other hand the total mass concentration of Hg should not be higher than is typical mass concentrations of total mercury in investigated waters. For natural water samples this means that higher enrichment of the $^{196}$Hg is better, because after addition of the spike into the sample, the total mass concentration of the Hg does not increase too high, i.e. it is still representing the routine samples. For example when total mercury mass concentration is 5 ng L$^{-1}$ (0.15% of that is $^{196}$Hg) in water sample, sufficient $^{196}$Hg spike would be for instance 3 ng L$^{-1}$. When we have mercury solution enriched with ca. 50% of $^{196}$Hg, we need to add total of 6 ng L$^{-1}$ mercury to achieve 3 ng L$^{-1}$ mass concentration for $^{196}$Hg in the spiked solution. The total mass
concentration of Hg in the same solution will be ca. 11 ng L$^{-1}$. For water samples with lower background level of total mercury concentration, either lower spike mass concentration or higher Hg enrichment is needed. The first option leads to the requirement of lower LOQ of the Hg measurement, while for the second option the higher price of the enriched isotopic material will be realised.

4. Conclusions

A new procedure of using the $^{196}$Hg isotope for the estimation of the stability of total mercury in water samples was presented. Experiments showed that the enriched $^{196}$Hg isotope can be successfully applied to the stability study. New information was obtained, since previously it was advised that the PT samples for total mercury measurement should be carried out within 14 days. In this study the tested samples were stable for more than three months, if they were stored at a temperature of 4–6°C. The results of this stability study were in line with the guidance presented in EPA standard 1631 [6]. However, the samples studied were noticed to be stable for a much longer time than is presented in the standard method ISO 17852 [5].

The stable nature of $^{196}$Hg is a benefit as there is no risk of radioactivity. The drawback is the limited availability and the high price of $^{196}$Hg isotope materials. Although just few milligrams of $^{196}$Hg isotope reagent is enough for spiking of several thousands of samples for stability studies. Another disadvantage is that without higher enrichment of $^{196}$Hg, the approach is not applicable to very low mercury mass concentrations (<1 ng L$^{-1}$), which may be found from sea water samples. There is also possibility that the spiked $^{196}$Hg did not behave in similar way than natural Hg isotopes.

Therefore stability study procedure would be improved if the spike could be added to the water sample in the field at the time of sampling. This would require the gravimetric preparation of some kind of capsule containing an exactly known mass of $^{196}$Hg isotopic material as well as preservation acid and BrCl oxidising agent. This would provide more confidence in that the spiked $^{196}$Hg behaves similarly as natural isotopes, since with BrCl it is expected to destroy and oxidise all organic mercury species to Hg$^{2+}$. Another option would be adding the enriched spike solution (by weighing) and BrCl to the bottle at the laboratory (before sampling). Then the sample would be preserved easily and immediately after sampling.

This kind of ‘quality assurance pill’ could also be useful in other measurements, where samples have long transportation and storage times or where the environmental conditions (light, temperature) are not optimal for sample storage.

Accuracy of the stability study procedure presented is mainly limited by the accuracy (or the measurement uncertainty) of the total mercury measurement procedure. As the expanded measurement uncertainty of the CV-ICP-MS method for the total mercury measurement is 10%, the uncertainties of the spike preparation and spike addition are insignificant. Therefore another improvement to the procedure presented could be the application of an isotope dilution method for mercury measurement [24–26]. This is known to improve the accuracy (trueness and precision) of the measurement result, which would lead to decreased day-to-day variation and therefore more reliable threshold values for storage time study and more reliable observations of out-of-range situations.

For the future, our interest is to apply this stability study procedure to different types of water, taking into account factors such as temperature, seasonal variation (winter/summer), sample vessel material and/or sample pre-treatment (e.g. filtration).
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Supplemental data

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