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Preconcentration and speciation analysis of mercury: 3D printed metal scavenger-based solid-phase extraction followed by analysis with inductively coupled plasma mass spectrometry

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ABSTRACT

A selective method for preconcentration and determination of methylmercury (MeHg) and inorganic mercury (iHg) in natural water samples at the ng L^{-1} level has been developed. The method involves adsorption of Hg species into a 3D printed metal scavenger and sequential elution with acidic thiourea solutions before ICP-MS determination. Experimental parameters affecting the preconcentration of MeHg and iHg such as the sample matrix, effect of the flow rate on adsorption, eluent composition, and elution mode have been studied in detail. The obtained method detection limits, considering the preconcentration factors of 42 and 93, were found to be 0.05 ng L^{-1} and 0.08 ng L^{-1} for MeHg and iHg, respectively. The accuracy of the method was assessed with a certified groundwater reference material ERM-CA615 (certified total iHg concentration 37 ± 4 ng L^{-1}). The determined MeHg concentration was below MDL while iHg concentration was determined to be 41.2 ± 0.5 ng L^{-1}. Both MeHg and iHg were also spiked to natural water samples at 5 ng L^{-1} concentration and favorable spiking recoveries of 88–97% were obtained. The speciation procedure was successfully applied to two lake water samples where MeHg and iHg concentrations ranged from 0.18 to 0.24 ng L^{-1} and 0.50–0.62 ng L^{-1}, respectively. The results obtained demonstrate that the developed 3D printed metal scavenger-based method for preconcentration and speciation of Hg is simple and sensitive for the determination of Hg species at an ultra-trace level in water samples.

1. Introduction

Mercury (Hg) and its compounds are highly hazardous pollutants, with organic Hg compounds generally more toxic than their inorganic counterparts (iHg). Methylmercury (MeHg) is the species of highest concern due to its bioaccumulation ability, affinity to macromolecules, and slow metabolism [1]. According to the United Nations Environment Programme's (UNEP) Global Mercury Assessment 2018 report [2], human activities have increased total atmospheric Hg concentrations by about 450% above natural levels, thus leading to Hg loads in some food webs reaching levels of concern for ecological and human health. Typical concentrations of total Hg in natural water are in the low ng L^{-1} range, with MeHg content ranging from only a few percentages up to 30–40% of the total Hg [3–5]. The speciation of Hg is essential, especially to understand the bioavailability and toxicity of MeHg to assess the health risks of Hg and to better understand its biogeochemical cycle [6]. Unfortunately, speciation analysis is often challenging due to extremely low concentrations of Hg in non-polluted waters.

Various Hg speciation analysis methods have been developed and reviewed in recent years [1,5,7–15]. The most common methods used for Hg speciation are chromatographic methods including gas chromatography (GC) and high-performance liquid chromatography (HPLC) hyphenated to highly sensitive elemental specific detection techniques such as cold vapor atomic fluorescence spectrometry (CV-AFS) and inductively coupled plasma mass spectrometry (ICP-MS). In addition to chromatographic methods, non-chromatographic methods can be used to separate Hg species based on the different chemical and/or physical properties [5].

Non-chromatographic methods use simple instrumentation and are more available than chromatographic ones. Additionally, they can offer shorter analysis time, lower cost, and sometimes even better sensitivity than a chromatographic approach [16]. Unfortunately, these methods have more limited separation power i.e., they are not applicable for the separation of many species [17]. Nevertheless, non-chromatographic

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speciation methods are a well-accepted way of speciation analysis for many elements with competitive limits of detection (LOD) [16,17]. Different non-chromatographic methods for the determination of Hg species in natural waters have been reviewed [1,5,11,18] and many of these techniques are utilizing solid-phase extraction for Hg preconcentration. As mentioned by Amde *et al.* [1], most of the non-chromatographic techniques are for iHg species alone and often have an insufficient limit of detection, exceeding the low ng L^{-1} range required for natural waters. Hence, the development of sensitive, simple, and multi-Hg species-oriented methods is highly desirable.

In our previous work [19], a 3D printed metal scavenger (3D-Thiol i.e., PA12-Thiol) for Hg preconcentration was prepared and applied for the determination of iHg from water samples with ICP-MS. The PA12-Thiol proved to be a very efficient adsorbent for Hg, so the focus of this study was to expand the method for speciation of Hg in natural water samples at ng L^{-1} concentration level. Experimental parameters including the sample matrix, effect of the flow rate on adsorption, eluent composition, and elution mode were optimized to achieve preconcentration and speciation analysis of Hg. The developed speciation procedure was tested on a certified reference material (ERM-CA615) and finally applied to natural water samples.

2. Material and methods

2.1. Reagents

High-purity water (18.2 MΩ cm) used throughout the study was prepared from an ELGA PURELAB Ultra water purification system (High Wycombe, Buckinghamshire, UK) and all solutions were made by dilution on a volume/volume basis. High-purity hydrochloric acid (ANALPURE, 34–37%) was obtained from ANALYTIKA, spol. s r.o. (Prague, Czech Republic) and p.a. hydrochloric acid (≥ 37%) from Merck (Darmstadt, Germany). Nitric acid (≥ 65%) and thiourea (ReagentPlus ≥ 99.0%) were purchased from Merck. A stock solution of iHg (10 mg L^{-1} in 5% HNO_3) was obtained from PerkinElmer (Waltham, MA, USA) and stored at 4 °C. The stock solution of MeHgCl (PESTANAL, analytical standard ≥ 98%, Merck, 310.8 mg L^{-1} as Hg) was prepared by dissolving the reagent in a small volume of methanol (BAKER ANALYZED, (Ultra) Gradient HPLC Grade, J.T. Baker, Deventer, Netherlands) and further diluted in 0.5% HCl and stored at 4 °C in an amber-colored glass bottle. Standard stock solutions of C, Fe, Ir, K, Mg, Na, and S (1000 mg L^{-1}, Pure) were obtained from PerkinElmer. Ca (1000 mg L^{-1}) was prepared from $CaCO_3$ (≥ 99.0%, Merck). NexION Setup Solution for ICP-MS instrument set-up (1 μg L^{-1} Be, Ce, Fe, In, Li, Mg, Pb, and U in 1% HNO_3, Pure Plus) was purchased from PerkinElmer. Certified reference material of groundwater (ERM-CA615) was from the Joint Research Centre, Institute for Reference Materials and Measurements (JRC-IRMM). SiliaMetS Thiol (i.e., Thiol) was used as a chemically active component in the 3D printed scavengers and was obtained from SiliCycle (Quebec City, Canada). Polyamide-12 (PA12) and polyamide-11 (PA11) printing powders were purchased from BASF (Ludwigshafen am Rhein, Germany). Only new 15 or 50 mL polypropylene centrifuge tubes or 10% HCl acid-washed volumetric flasks and glass bottles were used during the study.

2.2. Instrumentation

Total Hg determination was performed with a PerkinElmer NexION 350D ICP-MS (Waltham, MA, USA) in standard mode similarly as in our previous study [20] and the operational conditions are listed in Table 1. A four-channeled peristaltic pump (LabV1, Baoding Shenchen Precision Pump Co., Ltd., Baoding, China) with black/white 3.18 mm i.d. PVC peristaltic pump tubing (PerkinElmer) was used for the delivery of solutions through 3D printed metal scavengers.

Table 1
Operational conditions used for Hg determination by ICP-MS.

System/Operating parameter	Type/Value/Mode
ICP-MS	PerkinElmer NexION 350D
Sample introduction	ESI prepFAST 4DX
Washing solutions for ESI prepFAST	Rinse 1: A mixture of 0.75% HNO_3 and 2.25% HCl (p.a.), Rinse 2: 3% HCl (p.a.), Carrier/diluent: 3% HCl (high-purity)
Mode of operation	Standard
RF power	1600 W
Plasma gas flow rate	18 L min^{-1}
Auxiliary gas flow rate	1.2 L min^{-1}
Nebulizer gas flow rate	0.92–0.96 L min^{-1}
Nebulizer	PFA-ST
Spray chamber	Baffled, glass cyclonic (2 °C)
Detector mode	Dual
Scanning mode	Peak hopping
Isotopes	Hg as the sum of ^{200}Hg, ^{201}Hg, and ^{202}Hg, ^{184}W
Internal standard	^{193}Ir at 80 μg L^{-1} in 3% HCl
Dwell time	100 ms for Hg, 10 ms for ^{184}W and ^{193}Ir
Number of sweeps/reading	50
Number of readings/replicate	1
Number of replicates	3
Curve type	Linear through zero

2.3. Preparation of 3D printed metal scavengers

The 3D printed metal scavengers (PA12-Thiol or PA11-Thiol) were prepared by mixing PA12 or PA11 powder (~50 μm diameter) with 10 wt% of Thiol (3-mercaptopropyl-functionalized silica gel, 40–63 μm), respectively. The printing process was done using a Sharebot Snow-White SLS 3D printer as described in our previous work [19]. The 3D printing parameters used for PA12-Thiol are also described in our previous work [19]. For PA11-Thiol, the used laser power was 40–50% (of a maximum of 14 W), a laser speed of 2160–2560 mm s^{-1}, and a printing temperature of 175–177 °C. A layer thickness of 0.1 mm was used for all 3D printed filters. The filters were thoroughly cleaned from any unsintered powder. One or two filters were placed in a 10 mL syringe and washed with high-purity water.

2.4. Adsorption experiments

In all experiments, sample solutions were prepared in an appropriate volume (e.g., 45 or 500 mL), of which 5 mL was reserved for ICP-MS analysis to obtain the initial total Hg concentration. The remaining solution was then used for adsorption experiments. All 3D printed filters were conditioned with ~10 mL of high-purity water to prepare the filter, solvate the functional groups, and remove any unsintered printing powder [21].

2.4.1. Effect of the sample matrix on adsorption

Initial adsorption experiments were conducted using PA12-Thiol filters. To investigate the effect of different sample matrix compositions on both MeHg and iHg adsorption, 10 mL synthetic solutions (n = 2) containing 20 ng L^{-1} of MeHg or iHg with different concentrations of thiourea (0, 0.01, 0.05, and 0.12%) and HCl (0, 0.5, 3, 4, and 6%) were slowly passed through the PA12-Thiol filter without using a peristaltic pump (**A**: high-purity water, **B**: 0.01% thiourea, **C**: 0.12% thiourea, **D**: 3% HCl, **E**: 0.12% thiourea in 0.5% HCl, **F**: 0.12% thiourea in 3% HCl, **G**: 0.12% thiourea in 4% HCl, **H**: 0.12% thiourea in 6% HCl). For **A**–**F** and **H**, calibration was performed using 0.12% thiourea and 3% HCl in the sample matrix, and for **G** using 0.12% thiourea and 4% HCl in the sample matrix. The sample matrices were also adjusted to these concentrations before ICP-MS analysis. The Hg concentrations of the initial sample solutions and effluent solutions were analyzed by ICP-MS and adsorption percentages were calculated.

2.4.2. Support matrix of the 3D printed metal scavenger

The use of PA12 or PA11 in the supporting matrix of the 3D printed metal scavenger was investigated. The chemically active component (Thiol) and its amount were kept constant in all experiments. Filters were prepared as described in section 2.3. Adsorption tests were performed in the same manner as previously described in 2.4.1 and 0.01% thiourea was used in the sample matrix.

2.4.3. Improving the adsorption

The effect of different flow rates (5, 6, and 7 mL min $^{-1}$) on the adsorption of MeHg and iHg was investigated. Sample solutions of 40 mL (n = 4) containing either 20 ng L^{-1} of MeHg or iHg in 0.01% thiourea were passed through a PA11-Thiol filter with a peristaltic pump. It was also studied whether the adsorption of MeHg could be improved by placing two PA11-Thiol filters in the same syringe instead of one filter. A sample solution of 40 mL containing 50 ng L^{-1} of MeHg was passed through either one or two PA11-Thiol filters (n = 6).

2.5. Elution experiments

Preliminary elution experiments were performed with a PA11-Thiol filter using a peristaltic pump with the eluent volume of 10 mL at a flow rate of 0.5 mL min-1, followed by delivery of 2 mL of high-purity water. Experiments were performed in individual MeHg or iHg sample solutions at 50 ng L^{-1} concentrations (n = 2). The flow of the elution solution was in the same direction as adsorption in the preliminary experiments. In the optimized procedure (section 2.6), the flow of the elution solution (flow rate of 1 mL min^{-1}) was in the same direction as adsorption for MeHg, and counter-current flow was used for iHg without a peristaltic pump.

2.6. Optimized procedure

In the optimized procedure, the initial sample solution was adjusted to contain 0.01% thiourea, prepared in suitable sample volume, and a 5 mL of the solution was reserved for total Hg analysis. PA11-Thiol filters were conditioned with ~10 mL of high-purity water. The adsorption step was performed using a peristaltic pump and the sample solution was pumped through two filters at a flow rate of 5 mL min^{-1}. The retained MeHg species were eluted from the PA11-Thiol with two 5 mL aliquots of elution solution consisting of 0.1% thiourea in 3.5% HCl, followed by 1 mL of high-purity water at a flow rate of 1 mL min^{-1}. At the end of each elution step, the peristaltic pump was set at maximum speed for a brief time to recover the sample solutions from the filters.

Elution of iHg species was performed by countercurrent flow without a peristaltic pump. 2 mL of the elution solution consisting of 0.3% thiourea in 8% HCl was placed in a syringe holding the PA11-filters. The syringe plunger was used to push the first drop of elution solution through the PA11-Thiol filters and the rest of the elution solution was allowed to flow freely through the filters. Next, 2 mL of high-purity water was passed through the PA11-Thiol filters in the same manner. Before the washing step and at the end of the procedure, air was pumped through the filters with a syringe plunger to recover the sample solution. Individual sample solutions containing either MeHg or iHg species were adjusted to contain 0.18% thiourea and 3% HCl before ICP-MS analysis. Thus, the final volumes of the solutions containing either MeHg or iHg were 11.7 and 5.3 mL, respectively. The PVC tubes of the peristaltic pump used for sample delivery were washed with 5 mL of a solution containing 0.5% thiourea in 10% HCl at a flow rate of 5 mL min^{-1}, followed by 50 mL of high-purity water at a flow rate of ~50 mL min^{-1}.

2.7. Accuracy and precision

The analytical performance of the method was evaluated with replicate determinations of quality control (QC) sample (total Hg determination), and certified groundwater reference material of ERM-CA615 (n = 3, with and without speciation procedure). A natural water-mimicking QC sample was prepared by 10-fold dilution of QC stock solution (200 ng L^{-1} of iHg, 200 mg L^{-1} of C, 50 mg L^{-1} of Ca and Na, 10 mg L^{-1} of Fe, and 20 mg L^{-1} of K, Mg, and S in 30% HCl) and was adjusted to contain 0.18% thiourea and 3% HCl. For the analysis of certified reference material, 11,5 μL of 9.6% thiourea was added to 11 mL of ERM-CA615. As before, 5 mL of this solution was saved for total Hg analysis, and the rest of the solution was used for speciation. Speciation was performed as described in the optimized procedure (section 2.6).

2.8. Spiking studies and the treatment of the natural water samples

Water samples were collected either in 500 mL (spiking studies) or 2 L amber-colored glass bottles from Central Finland in April 2021. Sample bottles were cleaned as previously described [20]. Lake water 1 was collected from lake Jyväsjärvi (Jyväskylä) and lake water 2 from lake Päijänne (Korpilahti). Stream water was collected from Kor-ke-a-kos-ki (Jyväskylä). For spiking studies (n = 2), samples were spiked with 5 ng L^{-1} of MeHg and 5 ng L^{-1} of iHg, adjusted to contain 0.01% thiourea, and prepared to a volume of 45 mL. Natural water samples were prepared in triplicate to a sample volume of 500 mL. Samples were treated as described in the optimized procedure (section 2.6). Elution solutions were stored at room temperature overnight and analyzed by ICP-MS the following day. Considering the sample volume of 495 mL, the final volumes of 11.7 mL for MeHg and 5.3 mL for iHg, preconcentration factors of 42 and 93 are obtained for MeHg and iHg, respectively.

3. Results and discussion

3.1. Effect of the sample matrix on adsorption

The effect of eight different combinations of thiourea and HCl in the sample matrix was assessed on the adsorption of MeHg and iHg at 20 ng L^{-1} concentrations, and the results are shown in Fig. 1. Excellent adsorption (98–102%) was obtained for both MeHg and iHg with **A**, **B**, and **C**. However, when **A** was used in the sample matrix, the recovery of iHg in the initial sample solution was reduced to only 80%, indicating either Hg volatilization or possible loss of Hg by adsorption on the

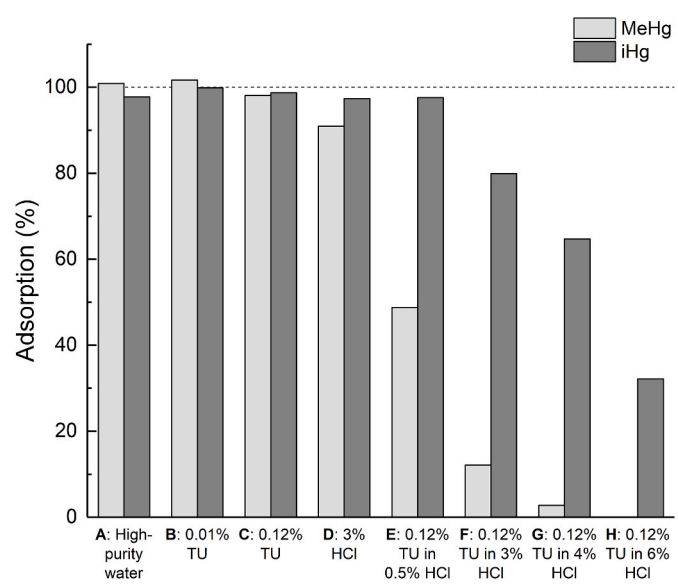

Fig. 1. The adsorption (%) of MeHg and iHg into PA12-Thiol using different combinations of thiourea (TU) and HCl in the sample matrix. The dotted line represents the target value of 100%.

container walls or the sample introduction system [22]. This was not observed with other sample matrices. When using matrix **D**, the adsorption of iHg was very efficient, but only about 90% of MeHg was adsorbed. It appears that iHg is more readily adsorbed than MeHg. Strong retention of iHg at lower pH could be explained as a higher affinity of Hg(II) towards –SH groups and the possibility for complex formation [23,24]. The retention of MeHg is often highly pH-dependent and the highest retention is usually achieved in the pH range of 4–8 [23,25,26]. Outside the optimum pH range, quantitative sorption of MeHg would be inhibited due to either its larger size, lower charge, and/or lower stability constant compared to iHg [23]. This also explains why low adsorption of MeHg was observed in matrices **G** and **H**, where higher HCl concentrations were used.

Indeed, other studies have aimed either to retain both forms of Hg [25] or retain iHg alone while MeHg is passed through the adsorbent [27–29]. For example, with an anion-exchanger material [29], iHg species are retained under selected conditions by the anion-exchanger as $HgCl_4^{2-}$, and they do not pass through the material. MeHg species, in turn, are passed through the ion-exchanger as MeHgCl [29]. It has been shown [30] that with thiourea-based coordinating resins, as the concentration of HCl in the feed solutions increases, the adsorption capacity of iHg is decreased. A similar effect was found in this study when PA12-Thiol was employed with a higher HCl concentration. It seems that the use of thiourea alone (**B** or **C**) in the sample matrix is the most appropriate as both Hg species are retained into PA12-Thiol. Therefore, **B** was used in further studies as this concentration of thiourea in the sample matrix is sufficient for the adsorption of both Hg species. Additionally, it is desirable to keep the total dissolved solids below 0.2% with ICP-MS [31], which also supports the choice of lower concentrations of thiourea. It should be noted that the effluent concentrations were in some cases below the limit of quantification (LOQ) and the obtained Hg concentrations have been used to calculate the adsorption percentages. Otherwise, significantly higher concentrations should have been used in all experiments, which does not reflect the natural state of the environment.

3.2. Support matrix of the 3D printed metal scavenger

The possibility of using PA11 in the supporting matrix instead of PA12 was investigated. PA12 is petroleum-derived while PA11 is a bio-based polyamide polymer made from castor oil, making PA11 a more sustainable alternative. The same amount of Thiol was used as a chemically active component in both filters. PA11-Thiol filters proved to be as efficient as PA12-Thiol filters, resulting in quantitative adsorption of both MeHg and iHg. The two polymers differ only by one carbon in the polymer backbone, but PA11 has been shown to have a better fatigue life than PA12 [32] thus being more durable. Due to the similar performance and lower environmental impact, PA11-Thiol filters were used in subsequent studies. Since the preparation of the metal scavengers was performed in the same manner as previously described and the macroscopic structure of PA12-Thiol has already been studied [19], this matter was not further investigated.

3.3. Optimization of the adsorption procedure

3.3.1. Flow rate

After finding a more sustainable supporting matrix for 3D printed metal scavengers, the effect of different flow rates on MeHg and iHg adsorption was investigated as described in section 2.4.3. It can be seen from Table 2 that iHg was more readily adsorbed than MeHg and the weaker adsorption of MeHg restricts the use of higher flow rates. Therefore, an adsorption flow rate was set at 5 mL min^{-1}.

Table 2
Adsorption (%) of MeHg and iHg at different flow rates using a PA11-Thiol filter.

Flow rate (mL min^{-1})	Adsorption of MeHg (%)	Adsorption of iHg (%)
5	96.1 ± 2.0	100.9 ± 0.7
6	93.7 ± 1.8	99.8 ± 0.7
7	89.4 ± 1.9	101.2 ± 0.4

Generally, SPE methods for preconcentration or speciation of Hg employ a sample flow rate of 1.5–5 mL min^{-1} [23,24,31–41], and the selected sample flow rate of 5 mL min^{-1} is at the higher end of this range. When using one PA11-Thiol filter, the adsorption of MeHg and iHg can be considered near quantitative.

3.3.2. Improvement of the adsorption of MeHg

It was examined whether the adsorption of MeHg could be improved by using two PA11-Thiol filters in the same syringe instead of one filter (section 2.4.3). When only one PA11-Thiol filter was used, in two of the six cases, the MeHg concentration of the effluent was above LOD (0.4 ng L^{-1}) but below LOQ (2 ng L^{-1}). By using two PA11-Thiol filters in the same syringe, the effluent concentrations of MeHg were below LOD. As two filters are placed in the same syringe, they are more tightly packed compared to just one filter. In our previous study [19], it was observed that relative standard deviation (RSD) values could also be improved if two filters were used instead of one, so two filters were used in subsequent experiments.

The PA12-Thiol has been shown [19] to be effective in adsorbing iHg with a maximum adsorption capacity of ~220 mg g^{-1} obtained from the Langmuir isotherm model. Because the adsorption capacity was found to be relatively large and far more than adequate for this purpose, and the chemically active component was not changed, the adsorption capacity was not further investigated in this study. In addition, if the adsorption capacity was to be determined, relatively high concentrations (several mg L^{-1} of MeHg) should be treated which is quite hazardous due to the high toxicity of MeHg.

3.4. Elution experiments

Acidic thiourea solutions have previously been used to elute iHg and/or MeHg from different adsorbents [19,24–26,35,44]. Krishna *et al.* [25] were able to elute MeHg alone from polyaniline using 10 mL of 0.3% HCl, followed by elution of iHg with 10 mL of 0.02% thiourea in 0.3% HCl. Mladenova *et al.* [23], in turn, used 2 mL of 4 mol L^{-1} HCl for MeHg elution from L-cysteine grafted silica gel and iHg was then eluted with 2 mL of 0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl. The concentration of thiourea and HCl used should be as low as possible considering the restrictions of total dissolved solids and acid concentrations with ICP-MS, while the volumes of sample solution and eluent, as well as the dilution of the sample solution, determine the preconcentration factor to be achieved. Since other studies have been successful in the sequential elution of MeHg and iHg, this approach was further investigated in this study.

3.4.1. Elution experiments focusing on MeHg

After optimizing the sample matrix and adsorption flow rate, elution tests were performed as described in preliminary experiments in section 2.5. When using only one PA11-Thiol filter, almost all MeHg could be eluted from the filter with the acidic thiourea solutions used. Unfortunately, also 9–38% of iHg was eluted from the filter (Table S1). To retain iHg more tightly in the 3D printed metal scavenger, two PA11-Thiol filters were used instead of one. This modification had obvious advantages for elution, as can be seen in Table 3. The retention of iHg was significantly increased, while good recoveries were obtained for MeHg. Of the eluents studied, 0.1% thiourea in 3.5% HCl was the only eluent that allowed elution of MeHg to be higher than 90% while retaining over 95% of iHg in the filter, so it was selected as the eluent for MeHg.

3.4.2. Improvement of the elution procedure

After MeHg is eluted from the filters, the next step is to elute iHg. In our previous study [19], 0.3% thiourea in 8% HCl was shown to be

Table 3
Recovery (%) of MeHg and iHg in preliminary experiments using two PA11-Thiol filters.

TU (%)	HCl (%)	Recovery of MeHg (%)	Recovery of iHg (%)
0.1	3	88.9	0.6
0.1	3.5	90.3	2.8
0.1	4	92.1	11.1
0.15	2	88.4	1.3
0.2	2	92.1	5.5
0.25	2	94.8	11.8
0.3	2	92.3	18.4

TU = Thiourea.

effective in eluting iHg, so it was also used as an eluent for iHg in this study. Elution of both MeHg and iHg was performed with their optimized eluents. The eluent volumes assessed for MeHg were 3, 5, and 10 mL and for iHg, these were 2, 3, and 5 mL. Elution was performed with a peristaltic pump in the same direction as adsorption. The flow rate of the eluent was increased from 0.5 mL min^{-1} to 1 mL min^{-1} and the washing step was omitted in this experiment. Good recoveries were obtained for MeHg with 10 mL eluent volume even if the flow rate of the eluent was increased as shown in Table 4.

However, unexpectedly low recoveries were obtained for iHg in this experiment. The low recoveries can be explained by the fact that the flow rate of the eluent was in the same direction as adsorption, so the elution was not as efficient if the flow was in the counter-current direction. To enable the separation of MeHg and iHg, the elution of MeHg was performed with a peristaltic pump in the same direction as adsorption, while the elution of iHg was performed counter currently in the following experiments as in our previous study [19].

Since two elution cycles are preferred to a single step [21], elution of MeHg was performed with two 4, 5, or 6 mL aliquots using the selected eluent at a flow rate of 1 mL min^{-1} followed by a washing step with high-purity water. As can be seen from Table 5, the highest recovery of MeHg was obtained with two 5 mL aliquots followed by a washing step with 1 mL of high-purity water.

These results suggest that selective and sequential elution could be performed if MeHg was first eluted from the filter in the same direction as adsorption, whereas the recovery of iHg could be significantly increased if eluted counter-currently [21].

3.5. Spiking studies

As the elution procedure described in the previous chapter seemed very promising, spiking studies were performed in different matrices as described in section 2.8. A total concentration of 10 ng L^{-1} of Hg was chosen for the experiments to have a similar concentration with natural water samples, but also to have a high enough concentration to make it possible to analyze Hg directly from the samples for comparison with the preconcentration method. The results are shown in Table 6.

When using high-purity water, an excellent recovery of 97% was obtained. Suitable recoveries (88–97%) were also obtained with natural water samples. The color of the water from both Lake Jyväsjärvi and Päijänne was slightly yellowish while Korkeakoski's water was brown-colored. The matrix of this brown-colored, humic-rich stream water might disturb both adsorption and elution steps, explaining slightly lower recovery. The results are consistent with the obtained total Hg concentrations from the direct ICP-MS analysis, confirming the applicability of the developed method for accurate and precise Hg speciation in natural waters at ultra-trace concentrations.

Table 4
Recovery (%) of MeHg and iHg from individual sample solutions using 0.1% TU in 3.5% HCl for MeHg and 0.3% TU in 8% HCl for iHg.

Eluent volume (mL)	Recovery (%) of MeHg with 0.1% TU in 3.5% HCl	Recovery (%) of iHg with 0.3% TU in 8% HCl
2	–	54.4
3	72.8	86.0
5	82.0	91.3
10	92.8	–

Table 5
Recovery (%) of MeHg using two aliquots of 0.1% TU in 3.5% HCl followed by washing step with high-purity water.

A total volume of the eluent + volume of high-purity water in the washing step (mL)	Recovery of MeHg (%)
8 + 2	74.6
10 + 2	87.3
10 + 1	94.0
12 + 2	90.6

Table 6
Results of the MeHg and iHg spiking studies in different matrices at a total concentration of 10 ng L^{-1}.

Sample	Elution of MeHg (ng L^{-1})	Elution of iHg (ng L^{-1})	Sum of the elution of MeHg and iHg (ng L^{-1})	Initial total Hg concentration (ng L^{-1})	Recovery (%)
High-purity water	5.4	5.0	10.4	10.7	97.4
Jyväsjärvi	5.6	5.9	11.5	11.8	97.2
Päijänne	5.6	7.0	12.6	13.3	94.4
Korkeakoski	5.8	7.6	13.4	15.2	87.9

3.6. Analytical performance and the analysis of natural water samples

The analytical performance of the proposed method was evaluated under the optimal experimental conditions. Linear calibration was performed using a blank, 10, 50, 250, and 500 ng L^{-1} of iHg in 0.18% thiourea and 3% HCl with a coefficient of determination higher than 0.999. LOD and LOQ for total Hg determination were estimated as the total Hg concentration corresponding to the average of 10 replicate measurements of the calibration blank plus three or ten times the standard deviation, respectively. The LOD and LOQ values were determined to be 0.4 and 2.0 ng L^{-1}, respectively.

To determine the method detection limits (MDLs) for MeHg and iHg using the speciation procedure, seven calibration blank solutions (500 mL) were taken through the speciation procedure as described in the optimized procedure (section 2.6) [45]. The elution solutions were prepared and analyzed over three non-consecutive days. To calculate the MDL for MeHg and iHg, the standard deviation of the blanks was multiplied by 3.14 (Student's *t*-test value for six degrees of freedom) and the preconcentration factors for MeHg and iHg were considered. The MDLs calculated for MeHg and iHg were 0.02 ng L^{-1} and 0.08 ng L^{-1}, respectively. However, considering the detection limits for total Hg determination, the MDL for MeHg was set at 0.05 ng L^{-1}, obtained by dividing the LOQ for total Hg determination by the preconcentration factor used for MeHg.

The RSD for ten replicate determinations of the QC sample within one day was 2.5%, and within four different days, the precision was 3.1% (n = 24). The obtained total Hg concentration for ERM-CA615 was 39.8 ± 1.3 ng L^{-1} with an RSD of 3.2% (n = 3). When the speciation procedure was utilized, MeHg was determined to be lower than MDL, while the iHg concentration was 41.2 ± 0.5 ng L^{-1} and an RSD of 1.2% (n = 3). The ERM-CA615 is originally acidified with HNO_3 to pH ~2 and spiked with inorganic $Hg(NO_3)_2$ to increase its Hg concentration to about 40 ng L^{-1} with a certified total Hg concentration of 37 ± 4 ng L^{-1}. Good recoveries for ERM-CA615 were obtained with (111.5 ± 1.4%) and without speciation procedure (107.6 ± 3.5%). According to the *t*-

test, no significant differences were found between the Hg concentrations using the speciation procedure and direct ICP-MS determination at the 95% level of confidence.

The proposed preconcentration and speciation procedure was applied to determine the concentrations of MeHg and iHg in lake Jyväsjärvi and Päijänne, as described in section 2.8. The determined MeHg and iHg concentration for lake Jyväsjärvi were 0.18 ± 0.01 ng L^{-1} and 0.62 ± 0.02 ng L^{-1}, respectively. Correspondingly, for lake Päijänne these concentrations were 0.24 ± 0.02 ng L^{-1} and 0.50 ± 0.02 ng L^{-1}. The results presented above demonstrate that the developed method enables Hg speciation at extremely low concentrations. The Hg concentration of the effluent solution was below LOQ in all studied samples.

The detection limits obtained in this study are considerably lower than in other similar solid-phase extraction studies for Hg speciation [23,26,33–35,37,39–43,46], with the exception of Krishna *et al.* [25], who achieved a LOD of 0.05 ng L^{-1} for iHg (Table S2). However, they used a more tedious method involving selective elution of MeHg and iHg from a homemade polyaniline column, UV irradiation for degradation of MeHg to iHg, CV generation, and trapping onto gold collector [25]. In the above-mentioned studies, the off-line preconcentration has also been performed by the flow-through process and the detection limits varied between 0.05 and 4300 ng L^{-1} for MeHg and 0.05–800 ng L^{-1} for iHg. Even lower detection limits have been obtained when online preconcentration has been utilized [3,38]. In addition, HPLC [33,34,37,39,41,42,46] or ion chromatography (IC) [38] have been commonly used to separate Hg species after preconcentration, which is not required in this study, simplifying the process significantly.

In our study, a 495 mL sample volume was chosen due to practical reasons such as ease of sampling and duration of the preconcentration procedure, which only takes about an hour and 40 min. The reusability of PA12-Thiol filters was studied previously [19] and the filters could be reused up to 10 times without a loss of adsorption efficiency. However, to avoid any contamination, only new filters were employed throughout the study. In our previous work [19], the preconcentration procedure is only applicable to iHg species, thus the method developed in this study is a significant improvement over the previous one. The method could further be improved by performing the preconcentration procedure in the field [11]. In this case, the loss of Hg species by evaporation and the risk of contamination from vessels would be minimized as Hg species are bound to the solid phase before transportation.

4. Conclusions

In the present study, a method has been developed and applied for the preconcentration and speciation of Hg in natural water samples at ng L^{-1} level offering an alternative to hyphenated techniques. The sample matrix is adjusted to contain 0.01% thiourea, which allows both iHg and MeHg to be adsorbed into a 3D printed metal scavenger. Selective and sequential elution is achieved by using 0.1% thiourea in 3.5% HCl for MeHg and 0.3% thiourea in 8% HCl for iHg. MeHg and iHg species, as well as the total Hg concentration, can be determined by the same ICP-MS method, which further improves the reliability of the developed method. Relatively high preconcentration factors of 42 for MeHg and 93 for iHg enabled extremely low MDLs of 0.05 ng L^{-1} and 0.08 ng L^{-1}, respectively. A comparison of the developed method with the previously reported solid-phase extraction methods for Hg speciation indicates that the proposed method is simpler than the existing methods and has an extremely low detection limit. The developed method can further help to understand the fate and toxicity of Hg species in aqueous systems.

Credit author statement

Suvi Kulomäki: Conceptualization, Formal analysis, Investigation, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. Elmeri Lahtinen: Conceptualization, Writing – review & editing. Siiri Perämäki: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. Ari Väisänen: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2021.123163.

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