# Simultaneous speciation and preconcentration of ultra trace concentrations of mercury and selenium species in environmental and biological samples by hollow fiber liquid phase microextraction prior to high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry

*F. Moreno*[a,b], *T. García-Barrera*[*a,b], *J. L. Gómez-Ariza*[a,b]

[a]Department of Chemistry and Materials Science, Faculty of Experimental Sciences, University of Huelva, Campus de El Carmen, 21007-Huelva, Spain

[b]Research Center on Health and Environment (CYSMA). University of Huelva. Spain

*CORRESPONDING AUTHOR:

e-mail: tamara@dqcm.uhu.es.

Tel.: +34 959 219962

Fax: +34 959 219942

ABSTRACT. Nowadays, hollow fiber membrane extraction techniques are widely used due to the high enrichment factors obtained with many different types of analytes and samples. In this paper, we propose a new analytical method that allows the simultaneous extraction of methylmercury, inorganic

mercury and $Se^{4+}$ and determination by high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The detection limits obtained are very low (110-230 ng/L) with relative standard deviations below 15% for all the analytes and averaged recoveries in fortified samples in the range of 71-99%. The precision of the analytical method is very good which overcomes one of the most important shortcomings of membrane extraction techniques. Several variables were studied to get optimal extraction conditions for the analytes. Method has been validated with real world samples as water (tap, river and estuarine) and human blood plasma.

KEYWORDS. Membrane extraction, hollow-fiber, water, ICP-MS, selenium, mercury, human plasma.

## 1. Introduction

Mercury is among the most dangerous substances for mammals, and due to the essential or toxic character of elements is species-specific, the main attention has been paid to the most toxic chemical compounds like methylmercury ($MeHg^{+}$). The most famous incident involving this element occurred in Minamata Bay where mercury compounds were dumped indiscriminately for decades causing important health problems to population. Mercury is usually methylated by benthic microorganisms (bacteria and sulphate reducers) [1], and it is bioaccumulated and biomagnified in the food chain.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA), which also evaluates chemical contaminants in the food supply, has established provisional tolerable weekly intakes (PTWIs) of total mercury at 5 μg/kg body weight, and at 1.6 μg/kg body weight of methylmercury [2]. The guideline level for total mercury in drinking water is established at 0.001 mg/L [3,4], and the European Commission Regulation 1881/2006/EC sets a maximum level of mercury at 0.5 mg/kg (fresh weight) in fish muscle meat and fishery products or, 1.0 mg/kg in some selected species (e.g. eel, pike, sturgeon).

On the other hand selenium is an essential element which is involved in several biological functions such as antioxidative action, regulation of thyroid hormone metabolism and cell growth [5]. Selenium is also incorporated to the active site of selenoproteins like glutathione peroxidase in organisms [6], and it has been proved the prevention activity of some selenium containing compounds against the development of diverse cancer types [7-9]. However, an excessive intake of this element has harmful effects in human health such as hair and nail loss, sickness, nerve damage nausea, and other harmful effects [6].

Nowadays, chemical speciation studies have generated very important information, and have led to the development of powerful analytical methods utilized in the fields of health, environmental issues and food quality control providing a wide application field. However, most of the methods are focused on only one element or very well-defined chemical species linked to an element, but some elements or their species can counteract the action of others through cooperation or availability mechanisms [10]. A good example is the antagonistic effect of selenium on mercury toxicity that was first reported in 1967 with an exposure experiment of rats treated with mercury chloride and selenite [11]; this protective effect of selenium against mercury toxicity has been reported by several authors [12-17]. To this end, analytical methods of multispeciation are necessary to deep insight into these biological, synergistic and inhibitory processes.

In addition, highly sensitive analytical methods are required for selenium determination since most drinking-water contains concentrations of this element that are much lower than 10 µg/L [18]. The United Kingdom Expert Group on Vitamins and Minerals recommended selenium intakes of 60 µg/day for women and 70 µg/day for men [19]. On the other hand, the United States National Academy of Sciences Panel on Dietary Oxidants and Related Compounds set an upper tolerable limit of selenium at 400 µg/day [20]. This level is also recommended by FAO/WHO (1998) [21] and the United Kingdom Expert Group on Vitamins and Minerals (EGVM, 2002) [19]. Therefore, highly sensitive speciation

methods are necessary to achieve the low levels of these elements (and their chemical species) in environmental or biological samples, and in this sense, hollow-fiber liquid phase microextraction (HF-LPME) introduced by Pedersen-Bjergaard and Rasmussen in 1999 [22] had become a very important procedure to preconcentrate many kind of analytes [23]. Miniaturised membrane based extraction techniques have several important advantages such as reproducibility, absence of sample carryover (due to the disposable nature of the membranes), high analyte enrichments, high throughput, low cost, applicability to many different types of analytes, large pH tolerance range and facility for automation and conversion into greener analytical techniques [24]. As a result, the development of analytical methods based on LPME has increased in the last years resulting in growing number of publications related with this topic with a 5-fold increased factor from 2005 to 2009 [25].

In the present paper, we describe a new analytical method for the simultaneous speciation of mercury and selenium species in environmental and biological samples (water and human blood plasma) using HF-LPME and high performance chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The method is very simple since only one chromatographic column and mobile phase is used to separate the species of both elements. The parameters of quality of the method are valuable and allow its satisfactory application to real world samples with high enrichment factors for the analytes, which in the case of selenium has been improved using a derivatization procedure.

# 2. Materials and methods

## 2.1. Standard solutions, reagents and samples

All reagents used for sample preparation were of the highest available purity. The solvents used as the organic phase in the LPME were toluene, chloroform, 1-octanol, bromobenzene, carbon tetrachloride, which were obtained from Sigma-Aldrich (Steinheim, Germany) and methyl isobuthyl

ketone (MIBK) that was purchased from Panreac Química S.A.U. (Barcelona, Spain). O-phenylendiamine used for selenium derivatization and 2-mercaptoethanol were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium selenite was obtained from Sigma-Aldrich (Steinheim, Germany), and methylmercury chloride, mercury chloride, hydrochloric acid and ammonium acetate from Merck (Darmstadt, Germany). Methanol was purchased from Teknokroma (Barcelona, Spain). Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water-purification system and was used throughout (Millipore, Watford, UK).

Aqueous stock standard solutions of selenium (1000 mg/L as Se) were prepared from sodium selenite ($Na_2SO_3$) in 2% (v/v) HCl solution. Methylmercury stock standard solution was prepared at 1000 mg/L (as Hg) by dissolving methylmercury chloride into 2% (v/v) $HNO_3$ solution. Inorganic mercury stock solution was prepared (1000 mg/L) by dissolving mercury chloride into 10% (v/v) $HNO_3$ solution. Working solutions were daily obtained by stepwise dilution of their stock solutions with high-purity de-ionized water. All standard solutions were stored at 4ºC in darkness until analysis. A tuning solution containing Li, Y, Tl and Ce (1 µg/L each) was purchased from Agilent Technologies (USA).

Samples were taken at the university (tap water), from a tributary of Guadiamar River affected by mining and agricultural activities (river water samples) and at the Odiel estuarine river also affected by mine lixiviates (estuarine water samples). Human blood plasma samples were provided from the Clinical Analysis Service of Juan Ramón Jiménez Hospital (Huelva, Spain). The investigation was performed after approval by the Ethical Committee of the University of Huelva.

**2.2. Instrumentation**

The HF-LPME assembly designed for the extraction of the analytes is shown in Figure 1. The porous hollow fiber used to support the organic phase was Q3/2 polypropylene (Accurel Q3/2, Membrana, Wuppertal, Germany) with an inner diameter of 600 µm, 200 µm of wall thickness and pores of 0.2 µm. The assembly consisted of two syringes model Hamilton 81330 (Hamilton Bonaduz,

GR, Switzerland), 15 mL vials (21 mm x 70 mm) (Supelco, Bellefonte, USA) and the previously described membranes. To carry out the derivatizations and extractions, a Selecta magnetic stirrer model Agimatic-N (Barcelona, Spain) equipped with a temperature sensor was used.

Chromatographic separations were performed in an HPLC system Agilent 1100 series (USA) equipped with a Rheodyne valve model 7725i (Bellefonde, PA, USA) and a 10 µL sample loop. The column used was a Phenomenex Luna C18 (250 mm x 4.60 mm, 5 µm, 100 Å pore size) (California, USA) and the outlet was directly connected to the nebuliser of the ICP-MS system by a PEEK tube. Elemental detection was performed using an ICP-MS model Agilent 7500ce (USA) equipped with a collision/reaction cell and a MicroMist nebuliser.

### 2.3. Derivatization method for selenium

A derivatization method described elsewhere [26,27] was used to form a piazselenol from $Se^{4+}$. Briefly, an aliquot of 50 mL of sample was taken, the pH was adjusted at 2 using a 2 M HCl solution, 1 mg of o-phenylendiamine was added and finally, the sample was held at 90ºC during 60 minutes.

### 2.4. Extraction of selenium and mercury species by HF-LPME

First of all hollow fiber was cut into appropriate length pieces (6 cm) which were used only once to avoid memory effects. Before using, each hollow fiber was sonicated for 5 min in acetone to remove any possible contaminant and later, the solvent was allowed to evaporate completely. For each experiment, 10 mL of sample (after derivatization) was placed into a 15 mL sample vial containing a 6 mm x 10 mm magnetic stirring bar. Two Hamilton syringes of 1 mL of capacity were fit to the ends of the hollow fiber, one of them was used to introduce the organic phase (bromobenzene) into the hollow fiber and the other one to collect the extract. The hollow fiber connected to the syringes was immersed in bromobenzene and then it was gently forced to penetrate into the pores and lumen of the hollow fiber to avoid the introduction of bubbles into the membrane and the incomplete filling of the pores and

lumen of the fiber with the extraction solvent. After that, surplus organic solvent was removed by contact with a cellulose sheet.

Finally, the hollow fiber was introduced into the sample vial adopting an U-shape configuration and a septum was fit to cover the vial and to support the syringe needles connected to the HF. Extraction was carried out during 20 minutes in a water bath with controlled temperature. Then, syringes and hollow fiber were removed from the sample and the inner volume extracted into one of the syringes which was injected in the HPLC loop (10 µL) for the analysis.

### 2.5. Speciation of mercury and selenium by HPLC-ICP-MS

The separation of the species by reversed phase HPLC was performed using a gradient of methanol (mobile phase B) and 0.14 % (v/v) 2-mercaptoethanol in 50 mM ammonium acetate at pH 4.6 (mobile phase A). The chromatographic conditions used for the separation of the species are collected in Table 1 and the instrumental settings of the ICP-MS detection are listed in Table 2. As can be seen, the isotopes $^{77}$Se and $^{82}$Se were monitored, but only the last was used for quantification in this work. A solution containing Li, Y, Tl and Ce (1 µg/L each) prepared in the mobile phase B was used to tune the ICP-MS for sensitivity, resolution, percentage of oxides and doubly charged ions. Typical chromatograms showing the separation of all the analytes are shown in Figure 2 (A-B) where the high enrichment factors attained after the extraction can be seen.

## 3. Results and discussion

### 3.1. Optimization of HF-LPME extraction

The most important variables that affect the HF-LPME extraction are the following: type of extraction solvent, HF length, immersion and extraction times, percentage of other organic solvent in the sample, stirring speed, temperature and ionic strength [28]. An univariate optimization of these variables was carried out, using the following initial set of values: temperature (25 ºC), agitation speed (700 rpm),

HF length (6 cm), immersion time (30 sec), ionic strength (0% NaCl), extraction time (5 min), percentage of other organic solvent in the sample (0%) and sample volume (10 mL). The optimization of the variables is shown in figures 3-8 where the relative peak area of the analytes (peak area/first peak area corresponding to the lowest experimental value of the abscissa) is represented in the ordinate axis.

Six different solvents were used for the extraction of analytes, namely: 1-octanol, bromobenzene, chloroform, MIBK (methylisobutylketone), carbon tetrachloride and toluene. As can be seen in Figure 3, carbon tetrachloride provides very good results for mercury species, but not for piazselenol, which can be extracted more efficiently using toluene and chloroform. A compromise situation for all the analytes can be attained with bromobenzene that was selected for further experiments. On the other hand, due to the low volatility of bromobenzene, possible losses during the extraction are reduced with respect to other solvents (with the exception of 1-octanol). Immersion time of 30 seconds in the solvent was considered enough to open the pores of the membranes because longer times do not improve the extraction yield.

The extraction process is based on analyte diffusion from the sample to the acceptor solvent inside the HF and consequently, an increase of the stirring speed may reduce the time to get the partition equilibrium. Stirring speeds from 0 to 1100 rpm were assayed and the results are shown in Figure 4. It can be observed that the optimal stirring speed which enhances extraction yields of all the analytes is 700 rpm that was selected for further experiments. Membrane length was optimized from 2 to 6 cm showing an increase of the extraction yields of all the analytes with membrane length (Figure 5). However, lengths higher than 6 cm were not used because the coupling with the extraction assembly is more difficult and finally, 6 cm of length was selected for further experiments. Although temperature has a positive effect on the extraction yields of the analytes, values higher than 60 ºC provoke important losses of the solvent contained in the lumen, and for this reason this temperature was selected for further experiments (Fig. 6).

It has been reported that an increase of the ionic strength affects the diffusion coefficients of the analytes [29-31] and for this reason the presence of NaCl in the sample was studied until 20 % (w/v). However, the presence of this salt has detrimental effects on extraction of both mercury and selenium species (Fig. 7) and was not used in further experiments. Finally, extraction time was optimized in the range of 5 to 40 minutes (Fig. 8). The extraction efficiencies increase with the extraction time, but an extraction time of 20 minutes was selected because longer times limit method applicability and sample throughput.

### 3.2. Parameters of quality of extraction method

Linear calibration curves were obtained from detection limits to 50 μg/L for all the analytes showing very good correlation coefficients (R> 0.994, in all the cases). The detection and quantification limits (LODs and LOQs, respectively) were calculated with the data generated in the linearity studies. After reshaping the calibration function, LOD and LOQ were calculated as the analyte concentration that corresponds to a signal equal to "a + 3 Sy/x" and "a + 10 Sy/x" respectively, where "a" is the origin ordinate, and "Sy/x" indicates the random errors for the slops. To evaluate the enrichment factor obtained with the HF-LPME assembly Eq. (1) was used, where $V_a$ is the volume of acceptor phase (0.015 mL), $V_d$ the volume of donor phase (10 mL) and $C_{eq\ a}$ and $C_{eq\ d}$ the concentration of analyte in the acceptor and donor phases, respectively.

The enrichment factors were calculated with solutions containing 5 μg/L of all the analytes and the results are presented in Table 3 along with the data provided by other authors for $Hg^{2+}$ and $MeHg^{+}$ [32,33], and $Se^{4+}$ [34,35]. We can observe that the enrichment factors are between 27 and 49 fold that is in good agreement with those published in the literature for the simultaneous extraction of $Hg^{2+}$ and $MeHg^{+}$ [33], while the Ef for the extraction of individual species as $Se^{4+}$ are lower [34,35]. However, these approaches do not consider the simultaneous extraction of Se and Hg species that may led to consider the results obtained with the proposed analytical method satisfactory.

Table 4 compares detection limits obtained with the present method and others previously published. As can be seen, detection limits improve the results for similar mixture of species without membrane preconcentration even for the analysis of single species as $Se^{4+}$. In addition, another important point is that the previously mentioned methods do not consider the analysis of multiple species of both elements.

### 3.5. Application to real world samples

The proposed method was applied to real world samples, namely: tap, river and estuarine water and human blood plasma. Only $Hg^{2+}$ was found in one sample from estuarine origin at 150 ng/L. To avoid the matrix effect, serum samples were ten fold diluted before the analysis. The matrix effect was evaluated by the comparison of the slopes of the calibration curves obtained by both external and standard addition calibration methods. Since a good correlation was observed between the slopes for all the analytes and samples, external calibration was selected for further experiments. Recovery experiments were carried out in samples fortified at three different concentrations 1, 5 and 25 µg/L and the results are in the range of 71-99 % (Table 5). As can be seen, the recoveries for all the analytes are very similar among the different samples showing a good performance of the HF-LPME assembly when it is applied to real matrices, such as water and biological samples. Precision was evaluated at three different concentrations (1, 5 and 25 µg/L) in tap water by performing repeatability (instrument and method precision), intermediate precision and reproducibility. The injection precision of the method was evaluated by performing ten replicate injections of the same sample extract. The relative standard deviation (% RSD) of the peak area was always below 0.4 % for all the analytes and concentrations, which was considered acceptable. The % RSD of the sample response factor was calculated for three separate extracts of each type of sample (human blood plasma, tap, river and estuarine water samples). The results ranged from 3 to 15 % for all the analytes that were considered acceptable at these low levels (Table 5). Intermediate precision was performed by two analysts, each testing three sample

extracts of three different fortified tap water samples on three separate days. Fresh sample and standard solutions were independently prepared on each day of analysis. The intermediate precision results ranged from 8-15 % for all the analytes and concentrations that was considered acceptable. Finally, the reproducibility of the method was calculated as the intermediate precision but in this case using two different HPLC and two chromatographic columns. The % RSD varies from 10-17 % that was considered acceptable.

## Conclusions

In this paper a new methodology for multispeciation of selenium and mercury is presented using hollow-fiber membrane extraction applied to real world samples. Since the detection limits of the proposed method are below those established by legislation in drinking water, it can be used in water quality control laboratories and moreover could help to understand the interactions between mercury and selenium in living organisms. The optimization has been performed to get the best conditions for the simultaneous analysis of all analytes under consideration ($Hg^{2+}$, $MeHg^{+}$ and $Se^{4+}$). The proposed approach provides detection limits in the range of ng/L and recoveries up to 71 % for all the analytes in different concentrations and matrices. Enrichment factors are in good agreement with other published methods that do not consider multispeciation of mercury and selenium but, the most important parameter of quality is the precision since with the proposed approach is possible to attain a %RSD in the range 3-15 % for all the analytes even in real complex samples as surface water and human serum. Finally, the approach is very simple and can be used for the routine analysis of mercury and selenium species in water and biological samples.

**ACKNOWLEDGMENTS**

The authors thank the Spanish “Ministerio de Educación y Ciencia (MEC)” project CTM2006-08960-C02-01/TECNO and the projects P08-FQM-03554 and P09-FQM-4659 from the “Consejería de

Innovación Ciencia y Empresa (Junta de Andalucía)" for funding.

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330

331 **FIGURE CAPTIONS**

332 **Figure 1.** HF-LPME assembly designed for the simultaneous extraction of selenium and mercury
333 species.

334 **Figure 2.** Chromatograms obtained from a solution containing 50 µg $L^{-1}$ of analytes before HF-LPME

(A) and after the extraction (B).

**Figure 3.** Effect of the solvent used for the HF-LPME in the relative peak area (n=3).

**Figure 4.** Effect of shaking speed used for HF-LPME on relative peak area (n=3).

**Figure 5.** Effect of the fiber length used for the HF-LPME in the relative peak area (n=3).

**Figure 6.** Effect of temperature on the relative peak area (n=3) in HF-LPME.

**Figure 7.** Effect of ionic strength on relative peak area (n=3) in HF-LPME.

**Figure 8.** Effect of extraction time on relative peak area (n=3) in HF-LPME.

## EQUATIONS

Equation I

$$Ef = 1 / (V_a / V_d + 1 / K_{a/d}); K_{a/d} = C_{eq,a} / C_{eq,d}$$

## TABLES

**Table 1.** Chromatographic conditions for the separation of the species

| Time (min.) | Composition (% A) |
|---|---|
| 0 | 80 |
| 2 | 44 |
| 8.2 | 44 |

A: 0.14% 2-mercaptoethanol and 50 mM ammonium acetate, pH 4.6
B: Methanol

**Table 2.** Optimal operation conditions for ICP-MS detection.

| | |
|---|---|
| RF Power | 1340 W |
| Plasma gas flow rate | 15 L/min |
| Auxiliary gas flow rate | 1.0 L/min |

| | |
|---|---|
| Carrier gas flow rate | 1.33 L/min |
| Sampling depth | 7 mm |
| Sampling and skimmer cones | Platinum |
| Dwell time | 0,3 s per isotope |
| Isotopes monitored | $^{77}Se$, $^{82}Se$, $^{202}Hg$ |

351

352 **Table 3.** Enrichment factors of the present method and others previously published

| Analytes | Ef | Reference |
|---|---|---|
| $Hg^{2+}$<br>$MeHg^{+}$<br>$Se^{4+}$ | 48<br>27<br>49 | Present method |
| $Hg^{2+}$<br>$MeHg^{+}$ | 760<br>115 | [32] |
| $Hg^{2+}$<br>$MeHg^{+}$ | 27.8<br>31.2 | [33] |
| $Se^{4+}$ | 480 | [34] |
| $Se^{4+}$ | 410 | [35] |

353

354 **Table 4.** Detection limits of the present method and others previously published

| Analytes | Matrix | Extraction procedure | LD (ng/L) | Instrumental method | Reference |
|---|---|---|---|---|---|
| $Hg^{2+}$, $MeHg^{+}$<br>$Se^{4+}$ | Tap water, river water, estuarine water and serum | - | 110<br>230<br>131 | HF-LPME-HPLC-ICP-MS | Present method |
| $Hg^{2+}$, $MeHg^{+}$<br>$Se^{4+}$ | Human urine and serum | - | 3000<br>10000<br>10000[1] | HPLC-ICP-MS | [36] |
| $Hg^{2+}$, $MeHg^{+}$<br>$Se^{4+}$ | Water standards | - | 3000<br>44000<br>72600 | HPLC-ICP-MS | [37] |
| $Hg^{2+}$, $MeHg^{+}$ | Natural freshwater | - | 11000<br>8000 | HPLC-UV-CV/HG-mAFS-AFS | [38] |
| $Hg^{2+}$, $MeHg^{+}$ | Liquid cosmetic samples | - | 1.3<br>7.2 | DLLME-HPLC-ICP-MS | [32] |
| $Hg^{2+}$, $MeHg^{+}$ | Water samples | - | 1.4<br>7.6 | DLLME-HPLC-ICP-MS | [39] |
| $Hg^{2+}$, $MeHg^{+}$ | Water samples | - | 3 | SPE-HPLC-ICP-MS | [33] |
| $Hg^{2+}$, $MeHg^{+}$ | Soil | 5 mL MeOH + 100 µL 6M HCl + 20’ MW | 35<br>73 | IC-CV-ICP-MS | [40] |
| $Hg^{2+}$, $MeHg^{+}$ | Soil and soil solutions | 9 mL HCl (7,6%, w/v) + 1 mL 2-M (10%, v/v) + 45’ US | < 15000 | DGT and RP-HPLC-ICP-MS | [41] |
| $Hg^{2+}$, $MeHg^{+}$ | Groundwater, waste water, sea water | - | 8-32 | RP-HPLC-ICP-MS | [42] |

| | | | | | |
|---|---|---|---|---|---|
| $Hg^{2+}$, $MeHg^{+}$ | Seawater samples, suspended solids | - | 0.07<br>0.02 | Online enrichment + RP-HPLC-ICP-MS | [43] |
| $Hg^{2+}$, $MeHg^{+}$ | Seawater and human urine | - | 150<br>350 | HPLC-CV-rf-GD-OES | [44] |
| $MeHg^{+}$ | Water | - | 0.02 | CV-AFS | [45] |
| $Se^{4+}$ | Serum samples | - | 670 | AEC-ICP-MS | [46] |
| $Se^{4+}$ | Infant formulas and dietic supplements | - | 400 | HPLC-CVG-AFS | [47] |
| $Se^{4+}$ | Urine | - | 800 | HPLC-MAD-HG-AFS | [48] |
| $Se^{4+}$ | Mineral waters | - | 450 | HPLC-UV-HG-AFS | [49] |
| $Se^{4+}$ | Cow milk | - | 400 | LC-UV-HG-AFS | [50] |
| $Se^{4+}$ | Water samples | - | 5 | HF-LPME-ETAAS | [34] |
| $Se^{4+}$ | Water samples | - | 7 | HPLC-ICP-MS | [51] |
| $Se^{4+}$ | Water samples | - | 0.5 | HF-LPME-ETV-ICP-MS | [35] |
| $Se^{4+}$ | Water samples | - | 30 | Coprecipitation-GFAAS | [52] |
| $Se^{4+}$ | Water samples | - | 10 | SPE-GFAAS | [53] |

2-M: 2 mercpatoethanol. MW: microwave. US: ultrasound. HF: hollow fiber. LLME: liquid-liquid microextraction. HPLC: high performance liquid chromatography. ICP: inductively coupled plasma. MS: mass spectrometry. UV: ultraviolet. CV: cold vapour. HG: hydrure generation. mAFS: modified atomic fluorescence spectrometer. AFS: atomic fluorescence spectrometer. DLLME: dispersive liquid-liquid microextraction. SPE: liquid phase extraction. IC: ion chromatography. DGT: diffusive gradient in thin films. RP: reversed phase. rf-GD: radiofrequency glow-discharge. OES: optical-emission spectrometry. AEC: anion exchange chromatography. MAD: microwave assisted digestion. LPME: liquid phase microextraction. ETAAS: electrothermal atomic absorpotion spectormetry. ETV: electrothermal vaporization. GFAAS: graphite furnance atomic absorption spectrometry.

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357 **Table 5.** Recovery experiments in fortified samples (% RSD, repeatability n=3).

**(a) Tap water**

| Analyte | 1 µg/L | | 5 µg/L | | 25 µg/L | |
|---|---|---|---|---|---|---|
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| Inorganic mercury | 82 | 11 | 75 | 8 | 76 | 9 |
| Methylmercury | 78 | 9 | 74 | 3 | 78 | 5 |
| Piazselenol | 73 | 11 | 82 | 14 | 74 | 12 |

**(b) River water**

| Analyte | 1 µg/L | 5 µg/L | 25 µg/L |
|---|---|---|---|

| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|---|
| Inorganic mercury | 90 | 11 | 86 | 12 | 88 | 12 |
| Methylmercury | 99 | 10 | 82 | 9 | 83 | 15 |
| Piazselenol | 87 | 12 | 93 | 8 | 95 | 10 |

**(c) Estuarine water**

| Analyte | 1 µg/L | | 5 µg/L | | 25 µg/L | |
|---|---|---|---|---|---|---|
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| Inorganic mercury | 75 | 14 | 85 | 11 | 73 | 10 |
| Methylmercury | 71 | 15 | 84 | 11 | 75 | 11 |
| Piazselenol | 82 | 6 | 95 | 10 | 81 | 11 |

**(d) Human blood plasma**

| Analyte | 1 µg/L | | 5 µg/L | | 25 µg/L | |
|---|---|---|---|---|---|---|
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| Inorganic mercury | 88 | 13 | 87 | 10 | 85 | 12 |
| Methylmercury | 84 | 15 | 99 | 11 | 95 | 10 |
| Piazselenol | 97 | 6 | 95 | 10 | 95 | 9 |

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