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## Journal Pre-proof

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**Highlights:**

- Simple, quick and cheap method for ultra-trace Hg quantification in natural waters
- Preconcentration of Hg on graphene nanoparticles and their direct analysis
- Excellent analytical performances: LOD = 0.38 ng L<sup>-1</sup> and reproducibility < 5 %
- Applicable for the analysis of both fresh and seawaters

Journal Pre-proof

# **A simple determination of trace mercury concentrations in natural waters using Dispersive Micro-Solid Phase Extraction preconcentration based on functionalized graphene nanosheets**

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## Abstract

In this work, we developed an innovative analytical method for the trace and ultra-trace determination of total mercury (Hg) concentration in natural water samples (fresh and seawaters). In this method, Dispersive Micro-Solid Phase Extraction (DMSPE) is applied using graphene nanosheets to quantitatively preconcentrate dissolved Hg from natural water samples, before its direct analysis by commercially available pyrolysis gold amalgamation and atomic absorption spectroscopy (AAS). In this new methodology, only two easy steps are necessary, saving time and effort. First, the operator has to add 500  $\mu\text{L}$  of nanoparticles suspension containing graphene, Ammonium Pyrrolidine DithioCarbamate (APDC) and Triton-X-100 in the water sample. This solution is filtered under vacuum and the Hg complex on the functionalized graphene can be simply collected on a membrane filter (Polyethersulfone PES, 0.2  $\mu\text{m}$ ). The filter obtained can then be analysed back at the laboratory by direct pyrolysis of the PES filter using a commercial mercury analyser. Different parameters have been tested to optimize this preconcentration procedure, such as the sample volume, the amount of nanoparticles suspension and the extraction time. The stability conditions of the Hg preconcentrated on PES filters during storage and before analysis has also been investigated. The influence of the occurrence of marine salts (sodium chloride), natural organic matter or competing metals (calcium) in the sample has also been evaluated to prevent possible matrix effects. This method is fully operational after application to real water sample matrices and exhibits suitable limit of detection, as low as 0.38  $\text{ng L}^{-1}$  using 200 mL of the water sample, and excellent reproducibility (< 5 % as RSD).

Key Words: Mercury; Preconcentration; Ultra-trace analysis; Graphene nanosheets; Dispersive Micro-Solid Phase Extraction (DMSPE); Water sample

## 1. Introduction

Among other pollutants, mercury (Hg) is of major concern in aquatic environments. While mercury is present at low concentration in natural waters [1], its specific biogeochemical cycling allows bioaccumulation and biomagnification in the food chain leading to serious consequences for animals and higher predators, including humans [2,3]. Consequences of mercury contamination are not only noticeable in terms of people's health [4,5] but also in the economic sphere [6,7] [9,10].

Thereby, mercury concentrations in topsoil, atmosphere and oceanic surface waters have increased respectively by a factor of 1.2, 4-6 and 3 since the pre-industrial period [8,9]. Besides, the numerous ecological studies about mercury show that primary anthropogenic mercury emissions are still increasing [8,10]. Therefore, the scenarios for the future are quite pessimistic, predicting a change in mercury emission of -4 % to 96 % [11].

Monitoring natural concentrations of total mercury in aquatic systems remains a difficult challenge since Hg is mainly occurring at ultra-trace levels: 0.3 to 8 ng L<sup>-1</sup> in waters free from local sources of mercury, either anthropogenic or natural, and 10 to 40 ng L<sup>-1</sup> for waters influenced by mercury mining and/or industrial pollution [12]. Indeed, a wide range of study has highlighted such levels of mercury in natural waters either seawater [1,9] or freshwater [13].

The development of low cost and easy handling analytical methods is then a necessity and it is required to fulfil some of the objectives of the Minamata Convention on Mercury, a global treaty to protect human health and the environment from the adverse effects of mercury. In that sense, Solid Phase Extraction (SPE) procedures to preconcentrate Hg before its analysis have shown a great interest in the scientific community due to its efficiency and simplicity. Most of the studies focus on the preparation of micro-column filled with specific adsorbents for Hg preconcentration. Polymers such as zwitterion-functionalized polymer microspheres (ZPMs) [14] or ion-imprinted polymer (IIP) especially designed for Hg adsorption [15] can be successfully used for rapid enrichment of mercury species. More simple in its preparation yet more expensive, the use of aminated Amberlite XAD-4 resin [16] or silica reversed-phase (RP-C<sub>18</sub>) [17] respectively followed by FI-CVG-AAS and GC/MS analysis allows

determination of Hg species, but its applicability to real samples remains difficult due to high limits of detection.

The investigation of new materials for Hg selective preconcentration made a great step forward in recent years. Indeed, due to their specific properties, nanomaterials have attracted increasing interest in the development of preconcentration methods for contaminants [18], and, more recently, Hg analysis. Then, the micro-column used to preconcentrate Hg can be packed with nanocomposites, previously functionalized for specific adsorption of Hg: magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticles [19], graphene oxide (GO) [20], multiwalled carbon nanotubes (MWCNTs) [17,21], carbon nanotube sponges (CNT sponges) [22]. Hence, all these methodologies include synthesis of the adsorbent, preparation of the column, elution of the Hg species directly followed by analysis, preventing direct application on the field. Moreover, multiplication of the preparation steps, from collection to analysis, may be problematic considering the potential contaminations or losses of Hg [23].

The use of field techniques reduces travelling and storage times of the samples, and facilitate the acquisition of results in developing countries and/or remote areas. In that sense, **K. Leopold et al.** [24–26] have conducted various studies in the last decade to develop a passive sampler for reagent-free on-site Hg preconcentration: nanogold-coated dipsticks. Thermal desorption of trapped Hg followed by AFS detection allows determination of Hg contents lower than the ppt level ( $\text{ng L}^{-1}$ ), and applicability to real samples, either seawater or freshwater, have been demonstrated. Nevertheless, implementation of this methodology in developing countries remains difficult because of the heavy preparation of the dipsticks (cleaning, coating, and functionalization) and their high cost (reagents and chemicals): sampling campaigns would need numerous of this passive sampler. Also, dipsticks have to be regenerated by thermal annealing at  $600\text{ }^\circ\text{C}$  for 20 min before each use, which greatly lengthens the preparation time of a sampling campaign. Finally, reproducible production of the nanogold-coated dipsticks remains difficult. As an alternative to expensive gold materials, **Tavares et al.** [27] have produced magnetite nanoparticles using iron oxide nanoparticles coated with silica shells functionalized with dithiocarbamate groups in order to take advantage of the strong affinities between Hg and thiol group (R-SH) [28,29] to preconcentrate Hg prior to its analysis by thermal decomposition AAS with gold amalgamation. Again, synthesis of the nanoparticles includes numerous steps and

reagents, and requires important scientific knowledge. Besides, extraction of Hg using these nanoparticles with recovery close to 100% necessitates at least 24 hours. Finally, the limit of detection of  $1.8 \text{ ng L}^{-1}$ , reached with this procedure prevents its application in pristine areas.

Several studies conducted by **Sitko et al.** have already been made using modified graphene nanomaterials for Dispersive Micro-solid Phase Extraction (DMSPE) such as carbon nanotubes [30], multiwalled carbon nanotubes (MWCNTs) [31], graphene oxide (GO) [32] or graphene nanosheets [33] in order to preconcentrate various elements (Mn, Fe, Co, Ni, Cu, Zn, Se, Pb, Cd). Nevertheless, none of these works deals with Hg, probably because of the high limit of detection of the apparatus (wavelength-dispersive X-ray fluorescence WDXRF, total-reflection X-ray fluorescence spectrometry TXRF, or energy-dispersive X-ray fluorescence spectrometry EDXRF) and the difficulties of working with such element (contaminations or losses).

In this work, we have used the specific properties of graphene nanosheets, a cheap material, to develop an innovative analytical method. Firstly, graphene is flexible so it can be easily attached to a support, which makes it a worthwhile candidate in the use of Micro-solid Phase Extraction (MSPE). It is as well hydrophobic and non-polar with a strong affinity for carbon-based ring compound due to the hexagonal arrays of carbon atoms in its structure. Graphene shows also a huge specific surface ( $2630 \text{ m}^2 \text{ g}^{-1}$ ) [34], which encourages its use as a sorbent in MSPE. It should be noted that this amazing material could be synthesized easily and affordably in most of the laboratories all around the world, including those of developing countries. Among graphene-based material, graphene nanosheets display great adsorption capacities. Indeed, both surfaces of a planar sheet of graphene are accessible to adsorption of analytes [35]. Also, nanostructured material as graphene nanosheets allows the adsorption of the organic compound via non-covalent interactions [36].

In the proposed method, based on **Kocot et Sitko** [33], dissolved Hg in water samples is first preconcentrated using a nanoparticles suspension consisting of graphene nanosheets as an adsorbent, Ammonium Pyrrolidine DithioCarbamate (APDC) as a chelating agent and Triton-X-100 as a surfactant. To obtain high recovery of the Hg on the nanoparticles, the procedure is optimized for various analytical parameters such as the amount of nanoparticles, sonication time and sample volume. The Hg-rich nanoparticles are then collected in a Polyethersulfone (PES) filter, whose stability



at different storage conditions is also checked. The potential existence of matrix effects due to the presence of chloride salts (estuarine or seawater), organic matter (continental water) and metals (calcium) able to compete with mercury for the binding sites of the sorbent is investigated, and the applicability of the whole method is finally verified by the analysis of real samples (seawater and lake water).

Together with the in-field preconcentration of a large volume of water on a small and light membrane filter and the direct analysis of this filter back to the lab greatly enhance the simplicity of all the analytical process for a precise and accurate determination of mercury content in water samples: from the sampling to the result!

## 2. Material and methods

### 2.1. Preparation of nanoparticles suspension

The principle of our method is based on the adsorption of mercury on graphene nanosheets. Nevertheless, metal ions in the form of hydrophilic complexes, including dissolved mercury, cannot be adsorbed directly on the graphene surface with a great efficiency [33]. Therefore, it is necessary to functionalize the graphene nanosheets using a chelating agent that will be adsorbed on the graphene nanosheets via non-covalent interactions ( $\pi$ - $\pi$  stacking) [35]. Ammonium Pyrrolidine DithioCarbamate (APDC) was chosen as a chelating agent due to some specificity in its structure. Indeed, strong affinities between APDC and mercury, mainly due to the presence of thiol groups (R-SH) in the structure of APDC, lead to the chelation of mercury by APDC (covalent bonds) [33]. -SH groups have already shown one of the strongest binding affinity either for inorganic mercury (sulphur-containing organic matter or polymers with Hg(II)) or organic mercury (sulphur-containing organo-metallic compounds or thiolate-like species with MeHg) [29]. We also need to add some Triton-X-100 in the nanoparticles suspension to increase the viscosity of the solution, hence improving the buoyancy of graphene nanosheets and, in this way, to obtain a real suspension of nanoparticles.

Then, following the recommendations of **Kocot and Sitko** [33], that applied DMSPE for the preconcentration of Co, Ni, Cu and Pb, the graphene/APDC/Triton-X-100 nanoparticles suspension was prepared as follows: 25 mg of graphene nanosheets ( $\sim 5 \mu\text{m}$  diameter, 1-5 nm thickness, Green Stone Swiss Co.), 80 mg of APDC (*Purum p.a.  $\geq 98.0\%$* , Sigma-Aldrich) and 10 mg of Triton-X-100 (*Laboratory-grade*, Sigma-Aldrich) in 20 mL of high purity water (18.2 M $\Omega$  cm), sonicated for 60 min, to obtain concentrations of 1.25 g L<sup>-1</sup>; 4 g L<sup>-1</sup> and 0.5 g L<sup>-1</sup> for graphene, APDC and Triton-X-100, respectively. To avoid the aggregation of the graphene and to ensure its homogeneity within the nanoparticles suspension, the solution was sonicated 5 min before each use.

## 2.2. Procedure for mercury preconcentration (optimum conditions)

The chelation between APDC and mercury can effectively occur only with a pH between 4 and 5, so initially, the pH of the water sample containing mercury was adjusted by the addition of ultrapure HCl and/or NH<sub>3</sub> solutions (*Optima grade*, Fisher Scientific). In the second step, 0.5 mL of nanoparticles suspension was added to either 20 mL or 200 mL of the aqueous solution to be analysed. Then, the solution containing the complex formed by Hg, APDC and graphene was sonicated for 5 min and collected on a membrane filter (*Polyethersulfone PES,  $\phi$  25 mm, 0.2  $\mu\text{m}$* , Pall Corporation) by filtration (250 mL Erlenmeyer glass flask) under vacuum. **Fig. 1** shows Transmission Electron Microscopy (TEM) images of water samples containing (a) graphene alone, (b) graphene and APDC, and (c) Hg, APDC and graphene. This figure really highlights an increase of the size in the observed particles from graphene alone (67 nm) to the complex Hg/APDC/Graphene (700 nm to 1.89  $\mu\text{m}$ ). Consequently, the size of this complex is larger than the membrane filter pores and is quantitatively retained by the filtering PES membrane. We noticed that the size of the complex Hg/APDC/Graphene remains lower than the certified commercial size of graphene nanosheets ( $\sim 5 \mu\text{m}$  diameter, 1-5 nm thickness). In our study, we assume that both the time in the ultrasonic bath (about 60 min) and the use of Triton-X-100 during the preparation of the nanoparticles suspension would promote the dislocation of aggregates, hence reducing the size in comparison with the raw graphene nanosheets [37].

Finally, the membrane filter obtained is dried for 5 min in a laminar flow hood (*Class 100*) and kept into polycarbonate filters holder (*Petri slides*) at low temperature ( $-7^{\circ}\text{C}$ ) before its analysis.

### 2.3. Instrumentation and method for mercury analysis

Direct pyrolysis gold amalgamation and atomic absorption spectroscopy (AAS), using an Advanced Mercury Analyser (*Altec AMA-254, SymaLab*), allows the quantification of Hg in solid samples. The whole membrane filter is dropped on a nickel boat especially designed for this instrument ( $1000\ \mu\text{L}$ , *SymaLab*). To increase analytical performances, nickel boats used for the analysis of the PES filters were previously cleaned by burning at  $750\ ^{\circ}\text{C}$  daily, using the AMA-254. This step has been repeated until a relative standard deviation under 10 % between two successive burnings is obtained. After the introduction of the sample into the analyser using the nickel boat, an increase in temperature up to  $750\ ^{\circ}\text{C}$  under oxygen atmosphere allows first the drying of the matrix, and then the decomposition of the sample and its dry mineralization. Decomposition products and mercury are driven by the oxygen stream through a catalytic tube continuously heated at  $550\ ^{\circ}\text{C}$ . This catalytic tube allows decomposition and reduction of methylmercury to gaseous elemental mercury  $\text{Hg}^0$  and the removal of halogens. Then, total mercury vapour is trapped in a quartz tube filled with gold-coated sand that allows the preconcentration of mercury, and so improve the sensitivity of the instrument. A few seconds later, the gold amalgamator is heated at about  $950\ ^{\circ}\text{C}$ . The quantification of this released mercury is carried out using two absorption cells (1 cm and 10 cm lengths, wavelength = 253.65 nm), both of them thermostatically controlled at  $125\ ^{\circ}\text{C}$ , that allows sequential quantification of mercury at two different ranks (Rank 1 < 25 ng Hg, and Rank 2 > 25 ng Hg).

All instrumental parameters have been optimized for analysis of PES filters as follow: drying step of 150 sec, decomposition step of 150 sec and waiting step of 45 sec. Accuracy and reproducibility of the AMA-254 were assessed by using the Certified Reference Material BCR-320R (channel sediment  $0.85 \pm 0.09\ \text{mg kg}^{-1}$  of total Hg), analysed regularly all along the measurement process according to the EPA method 7473 (SW-846).

### 2.4. Calibration for mercury quantification

To quantify the accumulated mass of mercury on the PES filter, the AMA-254 was calibrated using an external matrix-matched calibration curve in the absolute mass of mercury (2.3 – 207 ng Hg). Indeed, matrix effects can occur during the analysis of the PES filters and there is no existing certified reference material with a similar matrix. The Hg(II) standard solution (*Strem Chemicals, USA*) was prepared in 1% HCl with a concentration of 400  $\mu\text{g Hg L}^{-1}$  and various amounts were directly dropped into a nickel boat containing a PES filter resulting in absolute Hg masses of 2, 4, 10, 21, 31, 42, 53, 85, 144 and 207 ng. Here, we used Hg(II) considering the low fraction of MeHg in natural waters (< 10 % of total mercury) and assuming there would not be any difference in the adsorption process using either Hg(II) and/or MeHg (**Section 2.1.**). The absorbance (A) resulting from the analysis of unused filters was used for blank correction. The calibration functions obtained provide a reliable way to determine Hg content in the PES filters for both absorption cells, Rank1 ( $R^2 = 0.99947$ ) and Rank2 ( $R^2 = 0.99996$ ), with slopes of respectively ( $36.4 \pm 0.3$ ) and ( $560 \pm 1$ ) ng Hg AbsorbanceUnit<sup>-1</sup>.

The calibration curves according to the present procedure under the optimized conditions were also set up in the absolute mass of mercury (1.9 – 210 ng). Various samples containing 200 mL of high purity water were spiked with known amounts of Hg(II) standard solution ( $[\text{Hg}] = 400 \mu\text{g L}^{-1}$ ) resulting in absolute Hg masses of 2, 4, 8, 13, 19, 22, 43, 85, 148 and 211 ng. These samples were subjected to the preconcentration process previously described (**Section 2.2.**). The obtained calibration functions provide a reliable way to determine Hg content in the PES filters for both absorption cells, Rank1 ( $R^2 = 0.99974$ ) and Rank2 ( $R^2 = 0.99971$ ), with slopes of respectively ( $38.6 \pm 0.1$ ) and ( $576 \pm 3$ ) ng Hg Absorbance Unit<sup>-1</sup>.

Coefficients of determination  $R^2$  of the four different calibration curves are all above 0.99947, suggesting validated good linear relationship. Nevertheless, taking into account the standard deviations, the slopes given by these models are significantly different at a 95 % confidence level. This could be explained by the relative difficulty to weigh correctly the mass of Hg(II) standard solution added directly in the nickel boat in the case of matrix-matched calibration. Indeed, in practice, when the operator drops the solution into the nickel boat, the balance is not stable either due to adsorption on the nickel boat surface and/or evaporation. Therefore, in this work, we decided to use the

calibration curves obtained according to the procedure developed in this study under optimized conditions for data treatment.

## 2.5. Sampling of natural waters

To check the suitability of the proposed method to the analysis of natural waters, real samples were collected in April 2017 at two locations of the Pyrénées-Atlantiques (Nouvelle-Aquitaine, France). Freshwater was collected from Lac Des Carolins (43°20'15" N, 0°24'23" W), at a depth of 40 cm using acid cleaned polyethylene bottles (2 L) that were rinsed three times with the sampling water. Important wildlife in the surroundings of the lake has a great influence on its biogeochemical characteristics, especially concerning the relatively high content of organic matter measured in the sample ([DOC] = 5.6 mg L<sup>-1</sup>). Coastal seawater was collected in the same way out of the Bay of St Jean de Luz (43°23'37" N, 1°39'44" W, Bay of Biscay, North-East Atlantic Ocean). Time collection of the samples corresponds to high tide, and the seawater is characterized by a lower amount of organic matter ([DOC] = 0.90 mg L<sup>-1</sup>) and a high salinity (35.6 PSU). All water samples were filtered through a PES membrane (0.2 µm), acidified with ultra-pure hydrochloric acid (1 % v/v) and stored at 4 °C for no longer than two days before further manipulation and analysis.

## 3. Results and Discussion

### 3.1. Optimisation of the Hg preconcentration method

#### 3.1.1. Amount of nanoparticles

The amount of nanoparticles used in the preconcentration step will have a great influence on the efficiency of the proposed procedure. Enough nanoparticles must be available to chelate all the mercury present in the sample. Furthermore, an extra amount of nanoparticles will i) ensure that the possible presence of other competing metals will not negatively affect the effectiveness of Hg sequestration and ii) allow the use of a larger volume of sample to eventually improve the detection

limit of the method. In this work, the influence of the amount of nanoparticles has been studied. High purity water samples of 25 mL (n=4) were spiked with 100 ng of mercury (Hg(II) standard solution), and various amounts of nanoparticles have been added (expressed as volume of nanoparticles suspension added, from 100  $\mu$ L to 2000  $\mu$ L). Then, all the samples were subjected to the preconcentration procedure with a sonication time of 5 min. Blanks (n=4) were also produced for the various amounts of nanoparticles, following the same procedure with unspiked high purity water. The results are shown in **Fig. 2a.** which provides the mercury recovery obtained as a function of the amount of nanoparticles. No significant change was noticed in the recovery but the best condition selected was for a volume of nanoparticles suspension of 500  $\mu$ L, to ensure that all the mercury in the sample would be trapped even in the presence of a high amount of other competing metals. Indeed, a large amount of nanoparticles suspension leads to an increase in the blanks signal. The graphene shows similar properties to activated carbon so it can adsorb the gaseous species of mercury present in the air during the preparation of the nanoparticles suspension [38–40]. This could lead to the contamination in the blanks, making necessary to work under a clean atmosphere and to minimize the amount of nanoparticles suspension.

### 3.1.2. *Sonication time*

In previous works about preconcentration of metals using graphene nanoparticles as a sorbent, it is shown that the contact time between the nanoparticles suspension and the water sample does not appear as a critical variable as sorption occurs immediately [30,41]. Nevertheless, this parameter has been investigated here (**Fig. 2b.**) within the time range of 1-15 min in the ultrasonic bath. So, high purity water samples of 25 mL (n=4) were spiked with 100 ng of mercury, and preconcentrated using 500  $\mu$ L of nanoparticles suspension with various sonication times (1-15 min). Blanks (n=4) were also performed for each condition, following the same procedure with unspiked high purity water. Results depicted in **Fig. 2b.** show that sorption of mercury is significantly less effective when the time in the ultrasonic bath is lower than 1 min. Besides, for 2 min of sonication, the procedure shows a lower reproducibility (two replicates with about 87% of recovery and two replicates with about 100%

recovery) confirming that the sorption of mercury does not occur immediately. Finally, longer sonication times result in poorer reproducibility. This might be due to the influence of the ultrasonic bath that could degrade the non-covalent interactions ( $\pi$ - $\pi$  stacking) between graphene nanosheets and APDC. Therefore, sonication time of 5 min allows an effective and reproducible activation of the binding sites. For in-field direct application, we can imagine that the use of the ultrasonic bath can be easily replaced by a simple shaking, with longer time.

### 3.1.3. *Sample volume*

The volume of the water sample used during the method of preconcentration is one of the critical steps in the analytical performance. Indeed, the choice of a larger volume of samples leads to an increase in the preconcentration factor and, hence, to a decrease in the detection limits, provided that the nanoparticles present (same amount, lower concentration) can sequester quantitatively all the mercury that can be much more diluted in the medium. Calibration curves were obtained as described in **Section 2.4** using two different volumes of ultrapure water, i.e. 20 and 200 mL. **Fig. 2c and d.** show the absorbance obtained after the analysis of the filters produced according to the procedure described in **Section 2.2**, as a function of the theoretical amount of mercury added to the sample. Blanks ( $n=3$  for 20 mL and  $n=2$  for 200 mL of the sample) have been carried out for the two volumes, following the same procedure with unspiked high purity water. From 1.6 ng to 210 ng of mercury added to the test solution, no significant change can be observed in the efficiency of the preconcentration when working with 20 or 200 mL. A larger volume could have been tested, nevertheless, increasing the volume above 200 mL will be more time consuming for the operator in charge of the procedure because the filtration step will be much longer, especially with samples containing a high content of organic matter and/or suspended material. For future use of this method to quantify mercury in an aquatic environment, it is then important to adapt the sample volume to the specification of the water sample, especially when the concentration of Hg is very low.

## 3.2. **Optimization of the storage conditions**

The possible application of this preconcentration method directly in the field, in remote areas, implies the transportation of the filters from the sampling site back to the laboratory for their analysis. Thus, storage conditions of the filters is an important factor to take into account to minimize sample contamination as well as potential losses of mercury by evaporation. On the one hand, elemental mercury ( $\text{Hg}^0$ ) is present at trace levels in the ambient air. Typically, Total Gaseous Mercury (TGM) concentrations averages about  $1.5 \text{ ng m}^{-3}$  in background ambient air throughout the world [42]. In our laboratory, TGM concentrations have been reported by *Lusilao-Makiese et al.* [40] ( $6.3 \pm 1.6 \text{ ng m}^{-3}$ ), and are higher than the background ambient air levels due to the activities that take place in the laboratory (analysis and development of methodologies for metals and metalloids). This concentration, though, remains quite low for a laboratory building located in an urban area. Then, as  $\text{Hg}^0$  is subject to adsorption on all solid surfaces, it could eventually be adsorbed on the Hg-enriched filters produced in the preconcentration step and lead to an overestimation of the Hg content. On the other hand, the complex formed by Hg, APDC and graphene may be sensitive to degradation by light, temperature, oxygen, humidity etc... Then the exposure of the filters to various environmental conditions could lead to desorption of the mercury from the filters.

To avoid such problems, the stability of Hg-enriched filters has been evaluated using various storage conditions according to an isochronous design [43–45]. It is based on a storage design of the samples at various temperatures for different time intervals allowing all measurements to be done at the same time i.e. at the end of the study. In classical stability studies, measurements of the samples are achieved throughout the study i.e. at a different time so any drift of the measurement system over time can lead to incorrect conclusions. Thus, isochronous measurements only require repeatability conditions whereas classical stability studies require also long-term reproducibility.

The storage design set up in this study is shown in **Table 1**. Hg-enriched filters were obtained according to the present procedure under optimized conditions (500  $\mu\text{L}$  of nanoparticles suspension, 5 min sonication time), using 25 mL of high purity water samples spiked with 100 ng of mercury ( $n=3$ ). Then, the filters were kept into polycarbonate filter holders (Petri slides) and Zip-lock bags at the corresponding temperature for the required time. It has been assumed that mercury is stable on the filters at low temperature ( $-7 \text{ }^\circ\text{C}$ ). Thus, this reference temperature (i.e.  $-7 \text{ }^\circ\text{C}$  in a freezer) is the



temperature at which the samples were always transported or kept before the analysis and the testing temperatures (i.e. 4 °C in a cool room and 21 °C in a flow hood at ambient temperature) were the conditions at which test samples were stored for a selected period of time, before returning to the reference temperature. The total amount of Hg in all the filters was measured together at the end of the experiment.

To distinguish the various storage conditions of the filters and their efficiency, a Kruskal-Wallis test (nonparametric test), followed by a Conover and Iman test has been performed. **Fig. 3** summarizes the data collected in the isochronous measurement experiment along with the results of the Kruskal-Wallis test. On the one hand, after eight weeks of storage, the ratio between the recovery of the testing temperature T1 (21 °C) and the recovery of the reference temperature decreases significantly down to  $84.7 \pm 1.9 \%$ . On the other hand, after eight weeks, the decrease of this ratio with the testing temperature T2 (4 °C) is less important ( $93.2 \pm 1.4 \%$ ) but still significant. The only storage condition that does not show a significant difference with the results from the reference temperature is as follows: one week of storage at 4 °C. This allows the in-field application of the mercury preconcentration method as it is usually possible to keep the filters at low temperature (4 °C) in a portable fridge before coming back to the lab where the filters can be stored at low temperature (< -7 °C). If filters must be stored for a longer time, it is recommended to do it at freezing temperature, and even in that case, possible loss of mercury should be expected.

### **3.3. Potential interferences: matrix effects**

The potential occurrence of matrix effects due to the presence of interfering compounds in the sample was also investigated. For the sake of simplicity, the possible effect of salinity, organic matter and competing metals were modelled with NaCl, natural organic matter (NOM) and  $\text{Ca}^{2+}$ , respectively. Then, applicability to real samples with high organic matter content, and high salinity was demonstrated.

#### *3.3.1. Organic matter*

According to various studies about the adsorption of dissolved mercury and the influence of organic matter on this process, the efficiency of our new methodology could be eventually reduced by the presence of organic matter [46–48]. The reference material Suwanee River natural organic matter (SRNOM), purchased from International Humic Substances Society (IHSS, USA) was used to model organic matter. A solution with  $\text{NOM} = 500 \text{ mg L}^{-1}$  was prepared by dissolving 12.5 mg of NOM in 25 mL of high purity water. Firstly, high purity water samples of 25 mL ( $n=4$ ) were spiked with 100 ng of mercury, and various amounts of natural organic matter solution were added ( $[\text{NOM}] = 0 - 18.4 \text{ mg L}^{-1}$ ). Blanks ( $n=4$ ) were also produced in the same extent and under the same conditions with unspiked high purity water. The whole samples were preconcentrated using 500  $\mu\text{L}$  of nanoparticles suspension and 5 min sonication time. Recoveries in **Fig. 4a.**, calculated using the theoretical amount of Hg in the water sample and the amount of Hg obtained from the analysis of the Hg-enriched filter, show us that for  $\text{NOM} < 5 \text{ mg L}^{-1}$  there is no significant influence of the organic matter on the preconcentration of mercury on the nanoparticles ( $98 \pm 2 \% < R < 105 \pm 1 \%$ ). Nevertheless, for  $\text{NOM} > 5 \text{ mg L}^{-1}$ , the recovery decreases steadily down to  $84 \pm 3 \%$  for  $\text{NOM} = 18.4 \text{ mg L}^{-1}$ .

The potential influence of organic matter was also tested with real samples from Lac des Carolins ( $[\text{DOC}] = 5.6 \text{ mg L}^{-1}$ ). 200 mL of water sample from this lake ( $n=2$ ) spiked with a known amount of mercury (1.6 – 208 ng of mercury), and control solutions ( $n=2$ ) with the same amount of mercury in 200 mL of high purity water were simultaneously prepared. Then, samples were stored at room temperature, protected from light, in a laminar flow hood at least for 12 hours (equilibrium step). The samples were then treated according to the procedure described in **Section 2.2**. The percentage of controls, calculated as the ratio between mercury found in the real sample and mercury found in the controls for the same amount of spiked mercury (**Fig. 4c**) demonstrates that our new methodology for mercury preconcentration can be applied successfully to samples with a relatively high content of organic matter.

**Bravo et al.** [49], have studied the influence of organic matter on mercury species concentrations within 29 streams across Europe. In this study, total organic carbon (TOC) values range from 0.6 to 22.2  $\text{mg L}^{-1}$  and 76 % of the streams show  $\text{TOC} < 5 \text{ mg L}^{-1}$ , suggesting that our method is applicable to many freshwater systems.

### 3.3.2. Salinity

By analogy with organic matter, it has been shown that the presence of chloride salts might reduce the efficiency of the adsorption because of the formation of the resistant complex  $\text{HgCl}_4^-$  leading to an inhibition of the mercury adsorption on other sorbents [50,51]. The presence of chloride salts and their influence on the efficiency of the preconcentration procedure have been studied to determine applicability to seawater samples. Firstly, sodium chloride (*NaCl*, Merck KGaA, Denmark) has been added to high purity water samples of 25 mL ( $\text{NaCl}$ , 0 – 36 g L<sup>-1</sup>), also spiked with 100 ng of mercury. Replicates (n=4) of these synthesized solutions allowed us to produce Hg-enriched filters that have been analysed by AMA-254. Recoveries in **Fig. 4b.**, calculated using the theoretical amount of Hg in the water sample and the amount of Hg obtained from the analysis of the Hg-enriched filter, show us that for  $\text{NaCl} < 22 \text{ g L}^{-1}$  there is no significant influence of sodium chloride on the preconcentration of mercury on nanoparticles ( $100 \pm 1 \% < R < 107 \pm 1 \%$ ). Nevertheless, the recovery decreases slightly until  $93 \pm 2 \%$  for  $\text{NaCl} = 36 \text{ g L}^{-1}$ . For most natural waters, including groundwater, continental and estuarine waters, the presence of moderate amounts of salt should not lead to significant errors derived from the occurrence of matrix effects. Even in the case of high salinity seawaters, the effect should not be very pronounced (about 7 % suppression of the analytical signal).

To validate this hypothesis, we decided to apply the method of preconcentration to real samples with high salinity. 200 mL of water sample (n=2) from St Jean de Luz were spiked with a known amount of mercury (1.9 – 208 ng of mercury). Control solutions (n=2) with the same amount of mercury in 200 mL of high purity water samples were simultaneously prepared. After an equilibration step of 12 hours, Hg-enriched filters were produced according to the preconcentration procedure. The percentage of control, depicted in **Fig. 4d.**, demonstrates that our new methodology for mercury preconcentration can be applied successfully to samples with high salinity. Elsewhere, even if seawater contains high amounts of potential sources of exhaustion of the binding sites (sulphate, chloride, sodium, potassium etc...), the successful application of our new methodology to real seawater samples underlines the absence of competition of these ions with Hg for binding to the APDC.

### 3.3.3. *Competing metals*

With the variety of metal ions that could bind competitively with the chelating agent (APDC), calcium ( $\text{Ca}^{2+}$ ) is usually the major species present in water samples, and so it has been selected to investigate the competition with mercury for the chelation step. High purity water samples of 25 mL containing various amount of calcium ( $\text{Ca}^{2+} = 0 - 18.5 \text{ mg L}^{-1}$ ) were spiked with 100 ng of mercury. The ratio between results obtained for the reference filter (without calcium) and other conditions ( $R = 102 - 109 \%$ ) confirms that mercury is predominately bond to the APDC in all the conditions tested. Considering the strong binding affinity between  $-\text{SH}$  groups from APDC and Hg, a high competition or influence from the presence of other ions than  $\text{Ca}^{2+}$  is not expectable.

### 3.4. **Method blank levels and optimization**

Working under clean conditions is also a crucial consideration for successful analysis at very low concentration levels, in particular with mercury. In that sense, as mentioned in previous sections, for each change in a parameter during the preconcentration procedure (sample volume, nanoparticles suspension, sonication time, and amount of  $\text{Ca}^{2+}$ , NaCl and organic matter in the sample) replicates of high purity water were submitted to the same protocol. Low levels and good reproducibility for these blank samples lead to a very good limit of detection.

Firstly, to check any contamination before their use, unused PES membrane filters have been analysed using the AMA-254. The absorbance obtained was  $0.0025 \pm 0.0006$  ( $n=15$ ) and this value is not significantly different (t-test,  $p < 0.05$ ) from the value given by the analysis of empty nickel boats,  $0.0018 \pm 0.0002$  ( $n=9$ ), suggesting that no cleaning step is necessary for the PES filters.

Secondly, most of the experiments have been conducted in a trace metals laboratory whereas the last experiments, about real samples, took place in a clean laboratory, dedicated to ultra-trace mercury analysis. In the first cases, plastic flasks of 25 mL, cleaned in a 10%  $\text{HNO}_3$  bath, were used as a container and the blanks levels reached  $1.9 \pm 0.2 \text{ ng Hg per filter}$ . In the clean laboratory, special attention was paid to the procedure for cleaning all vessels used for sampling (polyethylene vials) and

sample preparation (glass vials, glass filtration system, and filter holders) with successive acid baths (10% HNO<sub>3</sub>, 10% HNO<sub>3</sub> and 10% HCl), reducing the blank levels down to 0.60±0.03 ng Hg per filter. Finally, it has been shown that graphene on its own can adsorb volatile Hg species, so it is probably responsible for the Hg content in the blank filters. This hypothesis was confirmed by the analysis of graphene directly by AMA-254: 0.82±0.01 mg Hg kg<sup>-1</sup>. One possibility to reduce such contamination, and therefore to reduce the blank levels, should be to buy new graphene nanosheets that will be managed only in a glove box under argon flux. Another solution could be to find a purification process not affecting graphene nanosheets structure and properties.

### 3.5. Analytical performances

The analytical performances of the method developed in this work are summarized in **Table 2** together with other methods for determination of mercury species at trace and ultra-trace levels. Traditional and/or newly developed methods highlight various problems preventing their application in-field and/or in developing countries: heavy sample preparation, high cost due to reagents and/or apparatus, and high limits of detection. Indeed, very sensitive analytical methods commonly used for the determination of Hg species, CV-AFS (LOD < 0.06 ng L<sup>-1</sup>) [52,53] and GC-ICP-MS (LOD < 0.04 ng L<sup>-1</sup>) [13], require a strong knowledge in analytical chemistry, and maintenance. Moreover, the use of these techniques implies a significant economic cost, due to either the equipment or the reagents. The use of a commercial mercury analyser for the analysis of the filters produced according to our new procedure greatly simplifies all the analytical process. Most of the newly developed methodologies focus on preconcentration of the Hg species followed by an elution step [15,17,20,54,55], making their application in the field laborious, if not impossible, which is not the case in our new method with the direct analysis of the filter. Finally, the most interesting published analytical methods for in-field Hg preconcentration need also strong knowledge in analytical chemistry for the preparation of the nanogold-coated passive sampler [26] and the magnetite nanoparticles [27] while the functionalization of the graphene nanosheets in our study appears much simpler. Moreover, in this work, just a few minutes are necessary to recover the whole Hg species on the functionalized graphene nanosheets

whereas **Tavares et al.** [27] need 24 hours. Overall, the novelty and the superiority of our work lie mainly on its simplicity all along its process: sampling (easily transportable filter), preconcentration (quickly and effortlessly producible adsorbent) and analysis (direct analysis of the solid without any elution step needed using a cheap equipment).

This new method for quantification of mercury by DMSPE using graphene nanosheets and direct analysis by pyrolysis gold amalgamation and atomic absorption spectroscopy (AAS) resolves many of these limitations and can be still improved. It provides a LOD as low as  $0.38 \text{ ng L}^{-1}$  for 200 mL of samples, with a large linear range ( $0.38 - 1038 \text{ ng L}^{-1}$ ). As concentration levels of total mercury in water samples (sea, estuarine, fresh) range in the  $\text{ng L}^{-1}$ , in most of the cases, our method is applicable, only with 600 mL of water sample for triplicate analysis. In the case of water samples presenting smaller mercury content, usually coming from pristine areas, such as ocean and many remote water bodies, the matrix is not complex, making the filtration step easier to manage. Therefore, for these particular cases, increasing the volume up to 1 L should allow a reduction of the LOD to match the Hg concentration in the sample.

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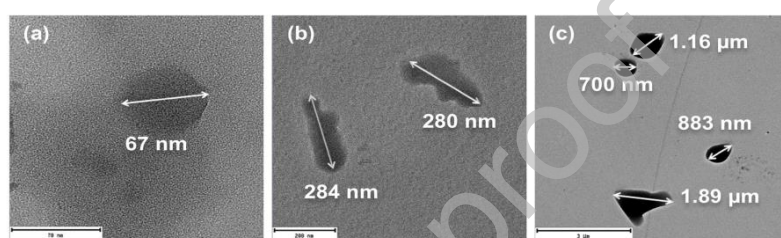
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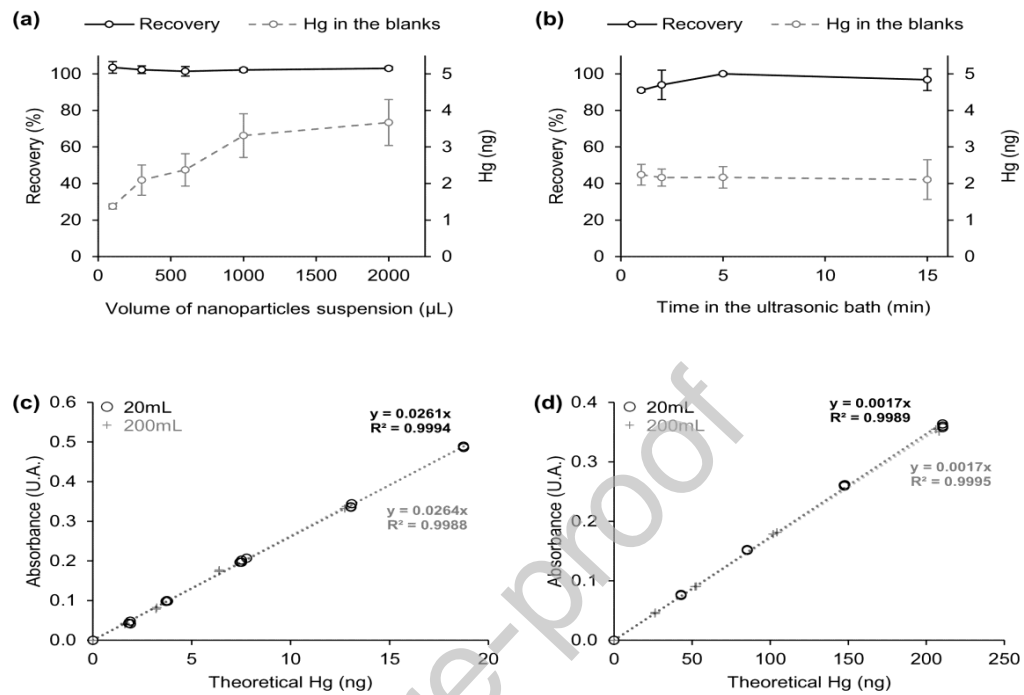
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**Fig. 1.** Transmission Electron Microscopy TEM of (a) Graphene, (b) Graphene + APDC and (c) Graphene + APDC + Hg(II)



**Fig. 2.** Recovery (n=4) of Hg (a) as a function of the volume of nanoparticles suspension and (b) as a function of the time in the ultrasonic bath; Theoretical Hg (ng) as a function of the absorbance given by AMA-254 to (c) Rank 1 and (d) Rank 2 for 20 mL and 200 mL of water sample

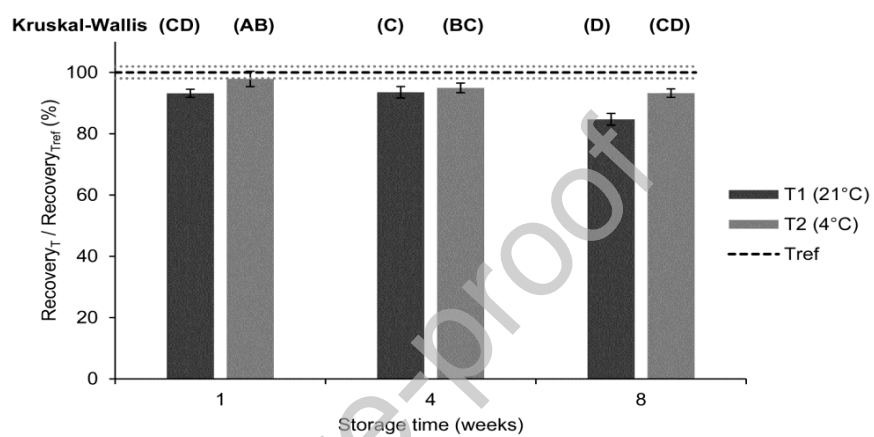
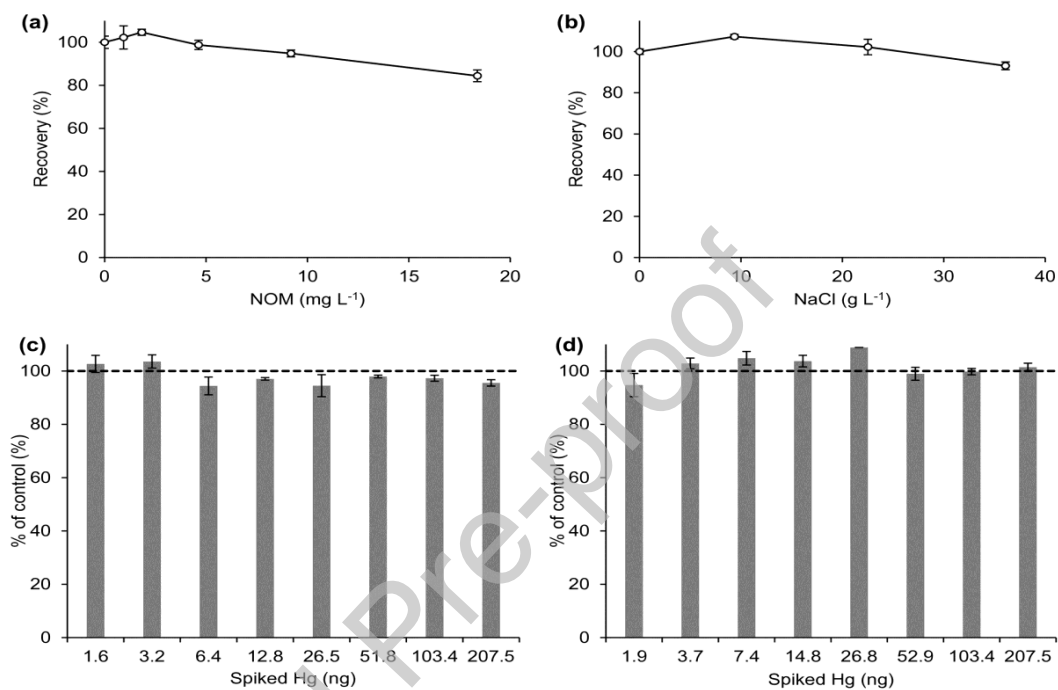


Fig. 3. Ratio between the recovery of Hg for the testing temperature T (n=3) and the recovery of Hg for the reference temperature T<sub>ref</sub> (n=3)



**Fig. 4.** Recovery of Hg **(a)** as a function of the concentration of natural organic matter (NOM) (n=4) and **(b)** as a function of the concentration of sodium chloride (NaCl) (n=5); Ratio (n=2) between control samples (high purity water) and real samples from **(c)** Lake Des Carolins (freshwater) and **(d)** St Jean de Luz (seawater) as a function of the spiked mercury



## Graphical Abstract

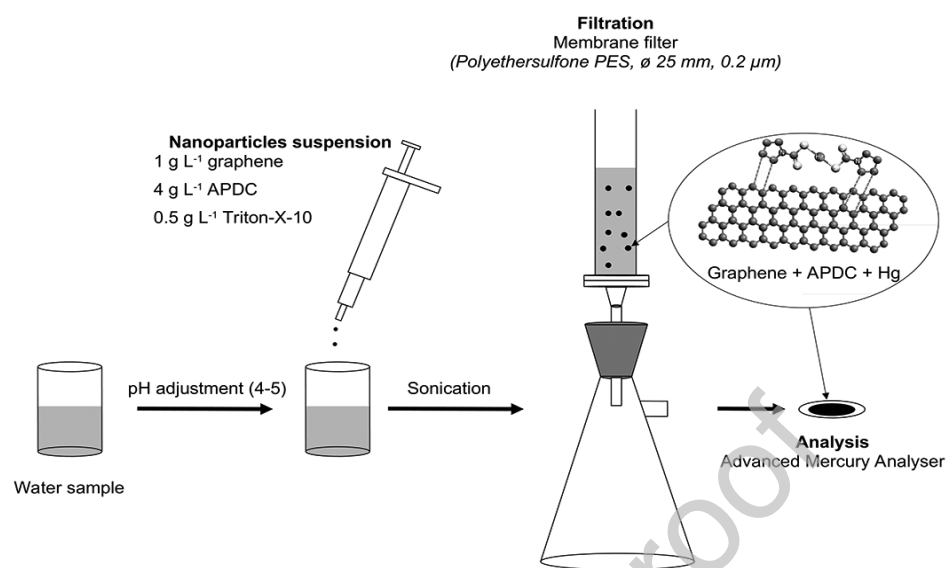


Table 1: Storage design for isochronous measurements to evaluate stability of the filters

Storage t (weeks) \ Temperature (°C)	0	1	2	3	4	5	6	7	8	9	10	11	12	
$T_1$ (21°C)		Storage at temperature T								Storage at very low temperature ( $T_{REF}$ )				
		Storage at very low temperature ( $T_{REF}$ )	Storage at temperature T							Storage at very low temperature ( $T_{REF}$ )				
		Storage at very low temperature ( $T_{REF}$ )				Storage at temperature T				Storage at very low temperature ( $T_{REF}$ )				
$T_2$ (4°C)		Storage at temperature T								Storage at very low temperature ( $T_{REF}$ )				
		Storage at very low temperature ( $T_{REF}$ )	Storage at temperature T							Storage at very low temperature ( $T_{REF}$ )				
		Storage at very low temperature ( $T_{REF}$ )				Storage at temperature T				Storage at very low temperature ( $T_{REF}$ )				
$T_{REF}$ (-7°C)		Storage at very low temperature ( $T_{REF}$ )												
Analysis		Storage at very low temperature ( $T_{REF}$ )												x



 Storage at temperature T  
 Storage at very low temperature ( $T_{REF}$ )

Table 2: Methods for analysis of mercury species in natural waters

Method	Volume (L)	Water type	Hg species	Quantification	RSD (%)	LOD (ng.L <sup>-1</sup> )	Note	Reference
Procedure presented in this work	0.2 (5min extraction)	Fresh and seawater	THg	External calibration	4.2 (Blanks) 3.0 (Standard) 3.2 (Fresh water) 4.5 (Seawater)	0.4	APDC/Graphene Nanosheets - DMSPE, filtration, and double gold amalgamation before AAS detection	This work
GC-ICP-MS	0.1	Fresh and seawater	Hg <sup>2+</sup> MeHg <sup>+</sup>	Isotopic Dilution (equilibration time 12h)	2.5 3.4	0.04 0.01	Alkyl derivatization to produce volatile species, extraction in GC organic solvent	[13]
CV-AFS (EPA 245.7)	0.25	Fresh water	THg	External calibration	n.d.	0.04	KBr oxidation, SnCl <sub>2</sub> reduction, purge of Hg(0), and AFS detection	[53]
CV-AFS (EPA 1631)	0.1	Fresh water	THg	External calibration	-5	0.06	BrCl oxidation, SnCl <sub>2</sub> reduction, purge of Hg(0), and double gold amalgamation before AFS detection	[52]
IIP-CV-AAS	0.1	Fresh and seawater	Hg <sup>2+</sup>	External calibration	2.4	0.5	Preparation of the IIP for Hg <sup>2+</sup> specific adsorption IIP - SPE column, elution by EDTA, and AAS detection	[15]
MWCNTs-GC-MS	0.025	Fresh and seawater	Hg <sup>2+</sup> MeHg <sup>+</sup> EtHg <sup>+</sup>	Internal standard quantification	6.2 6.8 7.2	4 3 3	Complexation of Hg species by NaDDC, MWCNTs - SPE column, elution by ethyl acetate with online alkyl derivatization	[17]
IL-GO-ETAAS	0.005	Fresh water	THg	External calibration	3.9	14	Preparation of the IL-GO hybrid nanomaterial for Hg adsorption IL-GO - SPE column, elution by 20% HNO <sub>3</sub> and ETAAS detection	[20]
NG-COOH-FI-CV-AAS	0.010	Fresh water	Hg <sup>2+</sup> MeHg <sup>+</sup> EtHg <sup>+</sup>	External calibration	<3	9.8	NG-COOH as solid-phase sorbent for US-D-IL-μ-SPE Elution by HNO <sub>3</sub> and analysis by FI-CV-AAS	[54]

<b>GO-ICP-MS</b>	0.010	Fresh water	Hg <sup>2+</sup> MeHg <sup>+</sup> EtHg	External calibration	4.5 3.1 3.7	0.005 0.006 0.009	GO as the SPE adsorbent Elution by benzoic acid	[55]
<b>AuNP-AFS</b>	n.d. (10min direct extraction)	Fresh and seawater	THg	External calibration	4.9	0.2	Nanogold-coated dipsticks (AuNP) - SPE Thermal desorption, double gold amalgamation before AFS detection	[26]
<b>NPs-AAS</b>	1	Fresh and seawater	THg	External calibration	<10	1.8	Fe <sub>3</sub> O <sub>4</sub> @SiO <sub>2</sub> SiDTC – DMSPE (24h extraction step), double gold amalgamation before AAS detection	[27]