Simultaneous determination of total and speciated mercury in marine sediment: a combined analytical approach

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Abstract: This study presents a combined analytical approach for determining total mercury (THg) and mercury species (CH_3Hg^+ and Hg^{2+}) in marine sediments**.** THg was measured using AMA-254, while speciation analysis was performed by GC-ICP-HRMS with double isotope dilution calibration approach. In the sediment sample, CH_3Hg^+ was 0.00054 ± 0.00011 mg kg^{-1} and Hg^{2+} was 0.118 ± 0.030 mg kg^{-1}. The sum of mercury species closely matched THg values obtained by AMA, confirming the method's reliability with 85% recovery. The low detection limits enabled accurate quantification of mercury, even at trace levels. Combining both techniques allowed a clearer distinction between the main mercury species and improved understanding of their distribution in the sediment. This approach is reliable for accurate mercury determination and the results can contribute useful information for future environmental assessments and monitoring efforts.

1 Introduction

Mercury (Hg) is a well-known environmental pollutant due to its toxicity and the ability to transform between different chemical forms depending on ambient conditions. One of the most hazardous species is methylmercury (CH_3Hg^+), which easily accumulates in aquatic organisms and participates in the food chain, eventually reaching top predators, including humans [1].

Marine sediments play an essential role in mercury cycling and its behavior is particularly complex. They can serve as temporary sinks, storing mercury for long periods. Under certain conditions they can also release it back into the water column, especially in its methylated form [2]. This methylation process is mainly driven by anaerobic microorganisms such as sulfate- and iron-reducing bacteria [3]. Factors like organic matter content, grain size of the sediment, sulfate availability, and microbial diversity directly influence of mercury transformation [2].

Environmental changes, such as low oxygen levels, physical disturbance of sediment, or climatic factors can promote the remobilization of mercury. All these factors making its behavior in the environment more difficult to predict and assess [1]. Although measuring total mercury (THg) provides useful information about contamination, it is the chemical speciation of mercury that determines its mobility and toxicity, which affects human health [4].

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Accurate quantification of mercury species is important, particularly because methylmercury and inorganic mercury (Hg^{2+}) differ significantly in toxicity and behavior [4]. Gas chromatography coupled with high-resolution inductively coupled plasma mass spectrometry (GC- ICP-HRMS) is one of the most commonly used technique for mercury speciation.

The analytical procedure includes extraction and derivatization steps that convert mercury species into volatile forms, suitable for detection [5,6]. To ensure accuracy, isotope dilution calibration (ID) is applied, which compensates for losses and matrix effects during the entire analytical process [6]. This approach allows sensitive and precise detection, even in complex matrices like sediment [5,6].

Exact identification of mercury species still remains a technical challenge, mainly due to possible changes during sample preparation, contamination risks, and the difficulty to account for all mercury forms present [6].

For total mercury quantification, the Advanced Mercury Analyzer (AMA) offers a simpler and efficient alternative to conventional digestion-based methods. The technique combines thermal decomposition, gold amalgamation and atomic absorption spectrometry. And also allows direct analysis of both solid and liquid samples with minimal risk of contamination. It is widely used in routine monitoring due to its speed, sensitivity and lack of chemical reagents. Still, some drawbacks remain, such as the limited sample size (up to 100 mg), the risk of memory effects and the need for careful instrument cleaning to ensure consistent results. And last but not least, the results depend on the homogeneity of the sample [7].

The present work demonstrates the benefit of combining results from AMA and GC-ICP-HRMS analytical techniques to produce reliable and precise data for mercury determination in marine samples. It shows how accurate and complementary analytical methods can help better understand mercury's complex behavior in the environment. Finally, this study points out the advantages of measuring both total mercury and mercury species, which supports a better assessment of contamination in marine systems. The main objective is to assess the performance of this approach through the analysis of a certified reference material and a real marine sediment sample.

2 Experimental

3.1 Instruments

2.1.1 Speciation analysis of Hg using GC-ICP-HRMS

For sample introduction and separation of mercury species (methylmercury and inorganic mercury) was used a Thermo gas chromatograph (Trace 1310) equipped with an RTx® - 5MS column (Crossbond® 5% diphenyl/95% dimethyl polysiloxane, 15 m × 0.25 mm × 0.25 µm). The gas chromatograph was coupled to an ICP-HRMS through a heated transfer line GCI 200 (Thermo Fisher Scientific, Bremen, Germany). The splitless injection mode was applied for a 2 µL sample volume. The injector and transfer line temperatures set at 250 °C. The GC oven temperature program was from 50 °C to 180 °C at 30 °C min^{-1}, with a hold time of 0.5 min. Helium was used as a carrier gas. The nebulizer gas flow was 0.498 L min^{-1}, and the scan mode was peak hopping.

2.1.2 Total mercury determination by the AMA 254 analyzer

Total mercury was measured using a mercury analyzer (AMA 254, Altech, Czech Republic). Samples and certified reference materials (CRM) were weighed into nickel boats and introduced into the instrument through an autosampler. After drying and thermal decomposition in an oxygen atmosphere, mercury vapor (Hg^0) was trapped on a gold amalgamator. Then released and detected

by atomic absorption spectrometry at 253.7 nm. Drying was performed at 220 °C for 60 s, decomposition at 850 °C for 150 s, and the measurement delay time was 45 s.

2.1.3 Reagents

All solutions were prepared using ultrapure deionized water (Milli-Q > 18.2 MΩ·cm, Millipore, Merck). Ultrapure hydrochloric acid (OPTIMA grade from Fisher Chemical, Seastar Chemicals Inc., Canada) was used for digestion of CRM and samples for methylmercury and inorganic mercury determination. A 1% sodium tetraethylborate solution was prepared by dissolving $NaBEt_4$ (Sigma-Aldrich, Germany) in deionized water; 1M sodium acetate buffer at pH 5 was prepared by dissolving sodium acetate trihydrate (VWR Chemicals, Belgium) and glacial acetic acid ≥ 99.7% (Sigma-Aldrich, Germany). Hexane (Merck, Germany) was used for derivatization in the determination of methylmercury and inorganic mercury by GC-ICP-HRMS. Glass screw-cap vials were used for liquid-solid extraction of CRMs and samples. Methylmercury solutions were prepared in dark glassware to protect from light.

Two certified isotope-enriched standards were used for spiking in the double isotope dilution method: ERM®-AE640 (enriched in ^{202}Hg) from EU-JRC-IRMM, Geel, Belgium, and a ^{201}Hg-enriched methylmercury solution from the Innovative Solutions in Chemistry (ISC-CH_3Hg^+), Oviedo, Spain.

Mercury(II) chloride of high purity (Sigma-Aldrich, Germany) and analytical-grade $HgCl_2$ salt (Alfa Aesar, GmbH & Co KG) were used to prepare individual stock solutions of mercury (1000 µg g^{-1}). Methylmercury chloride (Labor Dr. Ehrenstorfer-Schäfers, Germany) was used to prepare a 1200 µg g^{-1} stock solution in methanol and stored in the refrigerator. These stock solutions were used for preparing reverse isotope spike solutions in the double isotope dilution calibration. A standard with natural isotopic composition, ERM®-AE639 (EU-JRC-IRMM, Geel, Belgium), and CH_3HgCl (96% purity, C15100000 from Dr. Ehrenstorfer GmbH) were used for mass discrimination correction. All solutions were gravimetrically diluted as appropriate.

For method verification, a certified reference material was used: IAEA-158A Marine Sediment (Methyl Mercury) from IAEA Environment Laboratories (Monaco). The marine sediment sample was kindly provided by the Mediterranean Institute of Oceanography (MIO), Marseille, France. The originates from the West European coast. Detailed information on the original sampling procedure, such as the exact location, number of samples, collection method, depth, preservation, and storage conditions, was not available to the authors. The material had been collected and archived by MIO before this study. After receiving the sample, it was stored under laboratory conditions and applied standard pre-treatment procedures, including homogenization prior to analysis and drying for the mass dry correction.

2.1.4 Derivatization with Sodium Tetraethylborate

The following liquid-solid extraction procedure was applied: 0.3 g of CRM or sediment sample was mixed with 202Hg and $CH_3{}^{201}Hg^+$ spike solutions and 5 mL of 4 mol L^{-1} HCl. The mixture was stirred and heated at 60 °C for 20 min using a vial heater block. After cooling to room temperature, 5 mL of Milli-Q water, 5 mL acetate buffer, and approximately 2 mL of ammonia solution were added to adjust the pH to 4-5. Mercury species were simultaneously derivatized and extracted using 1.5 mL of Hexane and 500 µL of $NaBEt_4$. The samples were then shaken for 60 min using a vertical mechanical shaker at 210 rpm. The organic layer was transferred to GC vials, and 2 µL of each sample extract was injected into the GC-ICP-HRMS system. Instrumental blanks for GC-ICP-HRMS contained only Hexane, while procedural blanks included all reagents used in the full analytical procedure.

2.1.5 Total Mercury Determination with AMA 254

Total mercury was determined using the AMA 254, with internal calibration of the instrument. To ensure accuracy and monitor instrument drift, CRMs were measured between sediment sample runs. Appropriate sample weights were used: IAEA-158A (30-150 mg) and sediment samples (30-100 mg). A blank run (empty boat) was performed before starting measurements until the result was ≤ 0.3 μg L^{-1}, followed by alternating CRM and sample measurements.

2.1.6 Dry Mass Correction

Moisture content of the samples was determined by gravimetrically. Both CRM and sediment sample (0.44 g) were dried at 85 °C (±2 °C) until a constant weight was reached. The determined moisture content was used to correct the elemental concentrations to dry mass.

3 Results and discussion

Despite many studies and improvements in sample preparation for mercury species analysis, achieving accurate quantification remains difficult due to possible losses and transformations during pre-treatment [8]. Traditional calibration methods like external calibration or standard addition often fall short as they are unable to compensate for species-specific losses or changes, which may lead to inaccurate results. Additionally, relying only on certified reference materials during method validation is not enough to guarantee accuracy when interconversion between mercury species can occur [9]. Under such conditions, the isotope dilution calibration approach is more suitable. ID has been shown to improve accuracy in mercury speciation analysis while reducing measurement uncertainty [10]. In the double IDMS method, the concentration of the analyte in the enriched spike is first determined by reverse IDMS using a mixture of the spike and a high-purity standard. This step is crucial to accurately define the spike concentration for quantifying the analyte in the sample [11]. In this study, we applied a species-specific double isotope dilution calibration approach.

The mass discrimination corrections (K-factor) were essential to ensure the precision of ratio measurements. In this study, a bracketing calibration approach was used. To perform mass discrimination corrections for each sample was using a natural abundance solution. This practice effectively accounted for instrumental drift, ensuring precise ratio measurements. K-factor for CH_3Hg^+ for the ratio $CH_3{}^{201}Hg^+/CH_3{}^{200}Hg^+$ was estimated from 0.973 to 1.051 with 4.2 %RSD and for Hg^{2+} for the ratio $^{202}Hg^{2+}/^{200}Hg^{2+}$, K-factor was from 0.989 to 1.033 with 2.5 %RSD.

The precision of isotope ratio measurements was calculating from four consecutive determinations. The ratios $CH_3{}^{201}Hg^+/CH_3{}^{200}Hg^+$ and $^{202}Hg/^{200}Hg$ measured in a natural abundance mercury solution by ID-GC-ICP-SFMS were 0.5743 with 2.1 %RSD for methylmercury and 1.266 with 2.7 %RSD for inorganic mercury. The relatively high %RSD values of 2.1% and 2.7% are mainly due to instrument fluctuations at low analyte levels and matrix effects. These matrix effects occur when other substances in the samples interfere with the signal, either suppressing or enhancing it, which affects measurement accuracy. Together, these factors contribute to the variability observed in isotope ratio measurements for mercury speciation in complex samples like sediments [12].

The accuracy of the ID-GC-ICP-HRMS method was evaluated through recovery of enriched spike solutions used to spiked the samples and reverse spike isotope dilution samples. Six reverse spike isotope dilution calibration solutions were prepared to determine the precise concentrations of $CH_3{}^{201}Hg^+$ and $^{202}Hg^+$ spike solutions. Recoveries were 97% for $CH_3{}^{201}Hg^+$ at 1.135 ng g^{-1} and 102% for $^{202}Hg^+$ at 21.581 ng g^{-1}. The RSD values were 6.6% for methylmercury and 11% for inorganic mercury, both well within the acceptable limit of 20 %RSD for trace analysis

in complex matrices, recommended for confirmatory methods by the European Commission, Decision 2002/657/EU (2002).

In this study the CRM IAEA 158 were spiked with $CH_3{}^{201}Hg^+$ and $^{202}Hg^+$ before extraction procedure to achieve isotopic equilibrium. Rodríguez-González *et al.* [9] studies this approach during, which showed that full isotopic equilibration prior to extraction recovery of methylmercury concentrations in certified reference materials (DORM-2 and BCR-463) that closely matched certified values, with recoveries of 100.2 ± 4.0% and 96.5 ± 3.5%, respectively.

The results from the analysis of CRM IAEA-158 demonstrate the reliability of the mercury speciation method as shown in Table 1. The measured concentration of CH_3Hg^+ using GC-ICP-HRMS was 0.0012 ± 0.0002 mg kg^{-1}, closely aligning with the certified value of 0.0014 ± 0.0004 mg kg^{-1}, and recovery of 81%. For Hg^{2+}, the average concentration was 0.094 ± 0.028 mg kg^{-1}, compared to the certified value of 0.132 ± 0.014 mg kg^{-1}, with a recovery of 72%. These findings confirm that the method is well-suited for trace-level mercury determination in sediment matrices, particularly for CH_3Hg^+. The measured recovery of 81% for methylmercury falls well within the acceptable recovery range of 60–120% for analyte concentrations between 0.001 and 0.01 mg kg^{-1}, confirming the validity of the resultc in line with the UK Government marine sediment analysis guidance (2025).

The method's sensitivity is further demonstrated by its low detection limits. For CH_3Hg^+, the LOD and LOQ using GC-ICP-HRMS were 17 pg g^{-1} and 56 pg g^{-1}, respectively, while for Hg^{2+}, they were 12 pg g^{-1} and 38 pg g^{-1}. In comparison with AMA instrument used for THg determination showed an LOD of 3 pg g^{-1} and LOQ of 10 pg g^{-1}. These values were obtained following standard protocols for estimating detection limits [13]. For GC-ICP-HRMS, LOD and LOQ were determined from three and ten times the SD of the lowest concentration on the standard calibration curve, based on injections of 2 µL standard solution. Similarly, the LOD and LOQ of AMA were estimated based on the standard deviation from ten replicate measurements of blank cuvettes, performed under repeatability conditions. These detection capabilities confirm the suitability of the techniques for trace-level mercury determination in environmental samples. The previously published LOD and LOQ values are cited here to demonstrate the high sensitivity of the applied analytical methods, reported by the authors in another peer-reviewed journal [15].

As shown in Table 1. and Figure 1., the sediment sample contained an average of 0.00054 ± 0.00011 mg kg^{-1} CH_3Hg^+ and 0.118 ± 0.030 mg kg^{-1} Hg^{2+}. The sum of these species was consistent with THg levels, indicating efficient extraction and minimal mercury species transformations during sample preparation. It should be noted that other forms of mercury, such as ethylmercury or butylmercury, were not assessed in this study. The total mercury concentration measured in the sediment sample (0.138 ± 0.020 mg kg^{-1}) falls within the lower range of values reported in previous studies (0.05–1.5 mg kg^{-1}; Table 1 in Hellmann *et al.* [5]), indicating that the site is not significantly contaminated. Similarly, the measured CH_3Hg^+ concentration (0.00054 ± 0.00011 mg kg^{-1}) is at the lower end of the range typically observed in uncontaminated or moderately impacted sediments (0.0005–0.020 mg kg^{-1}; Hellmann *et al.* [5]), which is consistent with low methylation activity at the site.

Total mercury concentrations measured by the AMA 254 were 0.112 ± 0.019 mg kg^{-1} for IAEA 158 and 0.138 ± 0.020 mg kg^{-1} for the sediment sample. These values are slightly higher than the sum of CH_3Hg^+ and Hg^{2+} obtained through speciation analysis, resulting in a calculated recovery of 85% for both samples. This discrepancy falls within recommended limits (typically 70-120% for environmental samples by the European Commission, Decision 2017/848/EU (2017)) and may be due to matrix effects, volatilization losses, or incomplete recovery during derivatization. Still, the recovery aligns well with previously reported values in soils studies, which ranged from 102% to 104% [14].

The agreement between total mercury results from AMA and the sum of species measured by GC-ICP-HRMS confirmed the robustness of both methods. Although some losses or

transformations during the multi-step sample preparation were expected, the achieved recoveries showed that the double isotope dilution and optimized derivatization worked well to minimize them. The combination of AMA for fast analyzing and GC-ICP-HRMS for speciation analysis provided a reliable analytical basis for evaluating mercury contamination in sediment samples.

Table 1. Results for CH_3Hg^+, Hg^{2+} and THg in IAEA 158 and sediment samples analyzed with GC-ICP-HRMS and AMA, and their expanded uncertainties (k=2, norm.).

Sample	CH_3Hg^+	Hg^{2+}	THg (GC-HRICP-MS, sum)	THg (AMA)
		mg kg^{-1}		
*IAEA 158	0.0012 ± 0.0002	0.094 ± 0.028	0.095 ± 0.029	0.112 ± 0.019
Sediment	0.00054 ± 0.00011	0.118 ± 0.030	0.118 ± 0.027	0.138 ± 0.020

**IAEA certified value is 0.0014 ± 0.0004 mg kg^{-1} for CH_3Hg^+ and 0.132 ± 0.014 for Hg^{2+}*

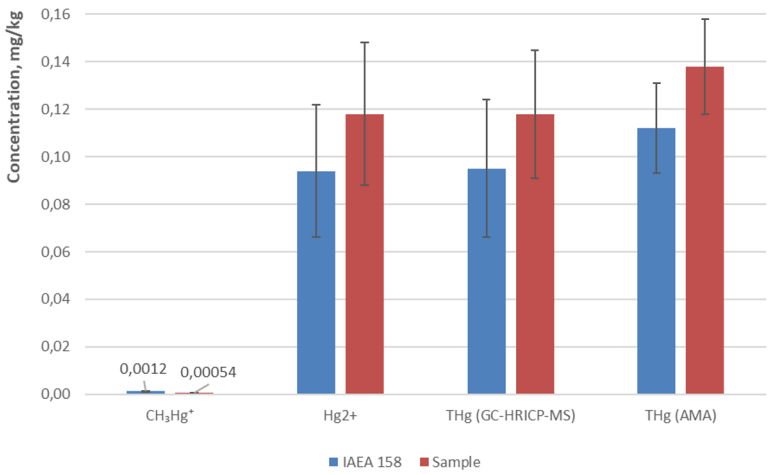

Fig. 1. Comparing mercury species (CH_3Hg^+, Hg2+, THg by GC-ICP-HRMS, and THg by AMA) in IAEA 158 and marine sediment sample

The maximum permissible concentrations of methylmercury and mercury in sediments (marine and freshwater), as established by national and international regulatory agencies. The Canadian Sediment Quality Guidelines (CCME, 2001) set limits of 170–486 µg kg^{-1} for fresh-water and 130–700 µg kg^{-1} for marine sediments. The Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG, 2018) recommend 150–1000 µg kg^{-1} for marine sediments. In Europe, the OSPAR framework (2009) defines a maximum value of 100 µg kg^{-1}. For methylmercury, the US Geological Survey (USGS, 2005) reports typical levels between 0.1 and 10 µg kg^{-1}, and up to 100 µg kg^{-1} in contaminated areas. The results showed good agreement between the two analytical methods. When applied to a real marine sediment sample, the combined approach confirmed that the total mercury content was below the established limits. Moreover, the main contributors to total mercury were inorganic mercury species, while methylmercury was found in trace level. This pattern is consistent with

uncontaminated or moderately impacted marine sediments, where methylation activity is limited. The low proportion of CH_3Hg^+ suggests that the potential for bioaccumulation and transfer through the food chain is limited under the current sediment conditions. It may also indicate low microbial methylation activity, possibly due to environmental factors such as redox conditions, organic matter content or sulphate availability [1,2].

The application of both techniques allowed for a better characterization of mercury contamination and demonstrated several analytical advantages. The AMA was given a quick total value for mercury, while GC-ICP-HRMS allows differentiation between toxic forms such as methylmercury and less harmful inorganic mercury. This strategy improved the interpretation of the data. In addition, this dual approach supports more accurate environmental risk and human exposure to mercury assessment. Moreover, it could help understand transformation processes in sediments. It also increases the reliability of analytical results by enabling internal verification of recoveries. Similar studies [19,20], also showed that combining methods gives a better understanding of mercury behavior in sediments. Although the future studies should examine seasonal variability, sediment depth profiles, and additional mercury species, such as ethylmercury and butylmercury. This would help to better understand transformation processes and potential remobilization. While this study shows the benefits of the approach, analyzing more mercury species would improve environmental assessment and provide more complete information.

3.1 Uncertainty

Evaluating measurement uncertainty is essential for establishing metrological traceability. This process involves careful identification and quantification of all main sources of uncertainty associated with both sample preparation and the analytical procedure, in order to develop a comprehensive uncertainty budget. The evaluation of measurement uncertainty was carried out according to widely accepted international guidelines, including the Guide to the Expression of Uncertainty in Measurement (JCGM 100:2008) and the FAO/WHO guidance document on measurement uncertainty (CXG 54-2004, updated 2023).

The overall uncertainty in the determination of mercury species using GC-ICP-HRMS primarily arises from four main sources: a standard deviation (SD) from 3 to 6 measurements of spiked CRM or sample, SD of 6 measurements of reverse spiked isotope dilution samples, mass fraction standard stock and spike solution, solutions mass and isotopes abundance.

Combined standard uncertainty of the results obtained by AMA was calculated from five main sources: a SD of 3-6 measurements of CRM or sample, a standard uncertainty of CRM from certificate, a standard uncertainty from recovery factor correction, a mass of CRM or samples weighted on a balance and a standard uncertainty from calibration curve associated with the second degree polynomial equation.

The expanded uncertainty was calculated using a k=2 (coverage factor) and 95% confidence level. The result was presented by formula: $U = 2.uc$, where uc was a combined standard uncertainty.

The uncertainty budget has already been established in a previous publication by the authors [15] and is applied here to the current measurements due to the similarity of analytical conditions and method validation approach.

4 Conclusions

This study demonstrates the benefits of combining AMA for total mercury with GC-ICP-HRMS for mercury speciation in marine sediments. AMA provides a quick and reliable measurement of

total mercury without acid digestion. At the same time, GC-ICP-HRMS, combined with isotope dilution, accurately detects methylmercury and inorganic mercury at very low levels. Certified reference materials and careful handling ensure the quality and traceability of the results. Together, the methods give a more complete understanding of mercury forms in sediments and support accurate environmental assessments. Although this approach does not capture all mercury species, it provides valuable data for future research and comparative studies.

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