Chromatography And Separation Techniques Journal

SCIENTIFIC LITERATURE

Special Issue Article "Extraction Chromatography" Review Article

Mercury Measurements in Environmental Matrices by ICPMS and HPLC-ICPMS: Nine Considerations

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ARTICLE INFO

Received Date: August 12, 2022
Accepted Date: September 16, 2022
Published Date: September 20, 2022

KEYWORDS

ICPMS
Mercury
Isotopes of mercury

Citation for this article: William A Maher, Frank Krikowa and Michael J Ellwood. Mercury Measurements in Environmental Matrices by ICPMS and HPLC-ICPMS: Nine Considerations. Chromatography And Separation Techniques Journal. 2022; 3(2):126

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ABSTRACT

Here we discuss our experiences with various approaches for measuring total Hg concentrations and speciation of Hg in environmental matrices. First, what Hg concentrations the method needs to measure should be considered as low Hg concentrations in samples will require pre-concentration before ICPMS analyses. Total Hg concentration measurements in waters require digestion with HNO_3-HCl to release Hg from particulate material and require a pre-concentration step to remove Hg from saline matrices and to obtain the required sensitivity. Freeze drying is required to remove moisture from plants and sediments as thermal drying procedures result in Hg loss. Biota analyses require the use of HNO_3 to extract Hg while sediments require the use of a HNO_3-HCl mixture to solubilise Hg and prevent adsorption to silicates or other insoluble components. Most samples will contain inorganic Hg2+ and CH_3Hg+ and occasionally CH_3CH_2Hg+. Volatile Hg species and those in water samples can be measured using hydride generation-trapping ICPMS and biota- sediment extracts using C8 or C18 HPLC-ICPMS. Extraction of Hg from biota-sediments can be achieved using cysteine or mercaptoethanol-based extraction reagents. HPLC mobile phases should be selected based on the elution order of the expected Hg species. In biota, CH_3Hg+ concentrations will tend to be much greater than Hg2+ concentrations, while in sediments, the reverse occurs. To prevent swamping of the smaller concentrations of Hg species, it should be eluted first. Hg adsorption to ICPMS components can be minimised by the use of cysteine in wash solutions and HPLC mobile phases.

INTRODUCTION

Understanding the environmental cycling and toxicology of mercury (Hg) has gained increased attention in recent years [1]. Environmental Hg problems first became apparent in the 1950's when methyl mercury (MeHg) was released into Minamata Bay Japan. Methylmercury was discharged in waste water from 1932-1968 from the production of acetaldehyde and vinyl chloride in which Hg was used as a catalyst [2]. "Minamata Disease' is now recognised as the chemical and pathological characteristics of the neurological disorders caused by Hg poisoning.

Mercury is mainly emitted to the environment from mining activities, the combustion of coal and industrial activities. Historically there have been gold rushes in Spanish America, the USA and Australia, all resulting in significant Hg inputs into the surrounding environments. Mercury can also enter aquatic environments through runoff from rock weathering, agriculture, wildfires, sewage and municipal wastewater [1]. It is estimated that 20% of anthropogenic Hg in the environment results from current

industrial activity while 60-70% is from legacy activities [3]. As well as total Hg concentrations, the speciation of Hg is required to understand Hg's mobility and toxicology. Mercury occurs mainly in inorganic forms as Hg^0 and Hg^{2+} in sedimentswhile CH_3Hg^+ have been reported in seawater, freshwaters, geothermal waters, sediments, landfill gases, fish, crustaceans, cetaceans and plants [1].

We have been analysing Hg in environmental matrices for over 40 years, and in this paper, we discuss our experiences in using Inductively Coupled Plasma Mass Spectrometry (ICPMS) to measure total Hg, Hg^{2+} and CH_3Hg^+ concentrations in waters, sediments, plants and fish tissues. We have structured the discussion around nine questions that should be considered before undertaking Hg analyses.

TOTAL Hg MEASUREMENTS

Question 1: How should samples be stored?

The factors contributing to the stability of Hg species in samples, especially water samples, are matrix composition, storage container, pH, temperature and light [4]. Generally, water samples need to be acidified to 0.1-1% v/v with HCl while biota and sediment samples need to be stored frozen or at a sufficiently low temperature to limit biological and chemical activity.

Question 2: What concentration of Hg does the method need to measure?

Total Hg concentrations are required to assess the distribution and movement of Hg in the environment. As well, accurate Hg analyses are required for speciation purposes to measure mass balances (extraction efficiencies, column recoveries etc.). The lowest Hg concentrations that can be typically measured routinely by ICPMS are around 0.010 µg/l using typical digestion mass of 0.1 to 0.2 g (dry mass) and an extraction volume of 10 to 20 ml. Using these conditions, the minimum tissue concentration is between 0.001-0.002 µg/g. For most water, plant and non-fish samples, Hg concentrations are too low for measurement without a pre-concentration step before measurement.

Question 3: Has Hg contamination in acids and other reagents been checked?

Mercury contamination is a widespread problem, and acids and other reagents often require purification before use. We have found that the use of a sub-boiling still is sufficient to purify HNO_3 and HCL while other reagents such as protease XIV, ammonium, cysteine and methanol can be purchased with sufficient purity, although batches must be checked for contamination before use. We have not experienced any issues with interfering elements such as W and Ta - elements that cause isobaric interferences in ICPMS.

Question 4: What is a suitable digestion procedure to release Hg from the matrix being analysed?

General considerations: Before solid samples such as biota and sediments are digested, samples need to be dried and ground to enhance solubilisation of Hg from matrices. We have found that the freeze-drying of samples prevents the loss of Hg during drying [5]. The literature has reported that the use of air or heat drying can result in the loss of Hg [4]. Some authors, however, state that sediment samples should be analysed fresh, i.e., not dried [6]. When grinding, care must be taken to ensure that the grinding process does not contaminate samples with W or Ta as these elements interfere in the measurement of Hg by ICPMS. We have found that agate ball mills or Tefcel (PTFE, glass fibre-reinforced) mills are suitable for this purpose.

The importance of sample particle size is often overlooked. Often methods are validated using CRMs that have well-characterised particle distributions (50-400 µm). The higher surface area to solvent contact for CRMs may result in better extraction recoveries than "real world" samples. Thus, we recommend the grinding of samples to at least 500 µm to ensure good extraction efficiencies.

Teflon digestion vessels are essential to minimise Hg adsorption and eliminate gaseous Hg exchange through container walls. All digestion and extraction vessels and polypropylene tubes needed to be cleaned by soaking successively in HNO_3 and HCL and after rinsing with deionised water dried in a laminar fume cupboard.

Water: Analysis of total Hg concentrations in water and wastewaters may require a pre-digestion step to release Hg from particulate material. In our laboratory, microwave digestion is used extensively with HNO_3-HCl (2:1 v/v); 0.1 mL of acid is added to 10 mL of sample at 150 °C for 30 min. If pre-concentration or separation from saline matrices is required, we use pre-concentration by a flow injection hydride generation system [7], leaving major salts in solution (Figure

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1A). Typical, only 5-10% of the solution enters an ICPMS during aspiration, whereas the use of a hydride generation increases the amount of Hg entering the ICPMS to 80-90%, thereby lowering the detection levels to ~0.0001 µg/l. Acid digestion of samples is not always necessary or desired; the aim may be to distinguish between particulate and dissolved Hg; thus, filtration, not digestion is required.

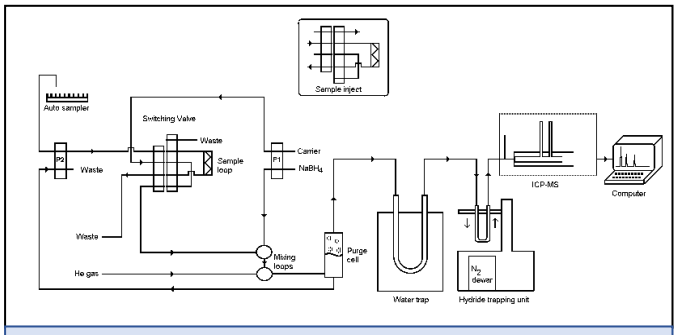

Figure 1A: Schematic of the flow injection-hydride trap-ICP-MS system. P1 and P2 refer to the peristaltic pumps of the FIAS-200 unit [7].

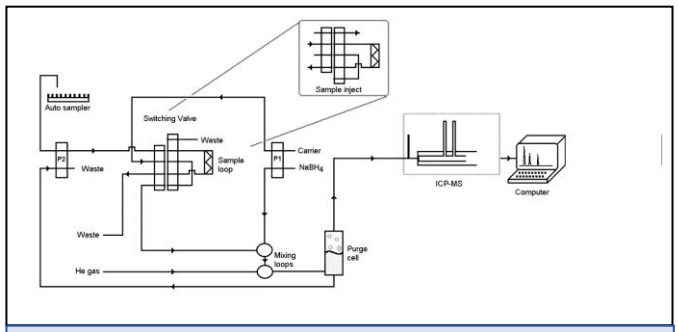

Figure 1B: Schematic of the flow injection-hydride ICP-MS system. P1 and P2 refer to the peristaltic pumps of the FIAS-200 unit (as optimized by Ragtap et al. [10]).

Biota: For the analysis of animal and plant tissues, most digestion procedures employ HNO_3 or HNO_3/H_2O_2 and microwave heating with closed vessels to quantitatively recover Hg. The use of closed vessel microwave digestion has been shown to reduce contamination and reduce mercury loss through volatilisation. Closed vessel microwave digestions also require less acid and eliminate the need to use perchloric or sulphuric acid that can cause problems in the ICPMS. Nitric acid is the preferred acid for use in ICPMS as it is relatively easy to purify and has few isobaric interferences. We routinely use 7 mL closed PFA-Teflon vessels to digest ~0.1 g of biological material with 1 mL of concentrated HNO_3 [8]. Predigestion at room temperature for 6-12 hrs is required to prevent rapid evolution of CO_2 at high temperatures and loss of Hg. After digestion; sample digests are diluted to 10 mL and 0.1 mL of concentrated HCl added if digests to be stored for long periods, Recoveries of Hg for certified reference materials have been shown to be in agreement with certified values

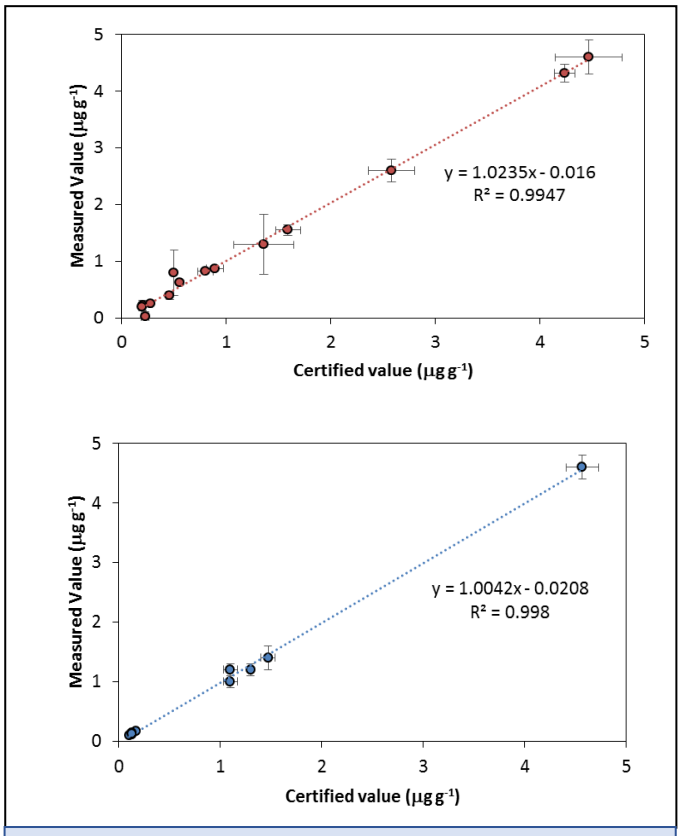

Figure 2: Measurements of Hg concentrations in certified reference materials (A) biota, (B) sediments and soils.

Sediments and soils: Evaluation of sediment extraction methods have clearly shown that using a mixture of HNO_3: HCL releases Hg from most sediment matrices, including those with HgS. We routinely use 50 mL closed PFA-Teflon vessels to digest ~0.2 g of sediment material with 3 mL of 1:2 v/v HNO_3 -HCL [9]. Again, predigestion at room temperature is required to prevent the rapid evolution of CO_2 at high temperatures and the loss of Hg. Recoveries of Hg for certified reference materials have been in agreement with certified values (Figure 2B). Normally the HPLC is directly coulped to the ICPMS. If more sensitivity is required, a hydride generation system can be used as described in Figure 1B, where the autosampler is replaced by the HPLC.

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MERCURY SPECIATION MEASUREMENTS

Question 5: Have you chosen the most appropriate speciation method?

The aim of speciation procedures is to maintain the integrity of Hg species and minimise sample preparation procedures that may alter Hg speciation.

Waters: Normally river and seawaters have low Hg concentrations and require pre-concentration of Hg species before analysis. The formation of volatile Hg species (Hg^0 and CH_3HgH), via the use of sodium tetrahydroborate (III) has been used successfully for water samples [10]. The efficiency of hydride generation is critically dependent on sample pH. We have described a fully automated hydride generation-trapping ICPMS system that satisfies these conditions (Figure 1A). We have found that the fully automated system with no glass components reduces contamination, i.e. lower blanks, and has much better reproducibility than batch systems. The trapping of Hg species critically depends on the chromatographic packing used in the trapping system, so particular attention should be given to this aspect of the system.

Biota and sediment: The main requirement of an extraction method is complete separation of the analyte from the interfering matrix without analyte loss, no sample contamination or changes in speciation. The most commonly used procedures for extraction of Hg species from samples are the use of microwave heating, sonication, distillation with acid, alkali or thiol-containing reagents such as 2- mercaptoethanol, thiourea or cysteine as extraction reagents [4]. Most of these procedures, however, are incompatible with HPLC-ICPMS as they use strong acids or bases that even after neutralisation, result in poor chromatography of mercury species. The use of microwave-assisted heating or the use of ultrasound has largely replaced conventional heating as it has advantages in terms of time, efficiency and solvent consumption. In microwave-assisted heating, the rapid heating of a solvent above its normal boiling point in closed vessels allows the extraction of samples in minutes. Parameters such as extraction medium, power applied, and heating time must be optimized, as well, the stability of the target species should be carefully evaluated. Better extraction efficiencies are achieved with little or insignificant sample loss while permitting easier and safer operation.

Since Hg has a high affinity for sulfhydryl groups, many studies have used sulfhydryl reagents as the extractant [4]. The use of S containing chelating agents such as cysteine has proved successful in extracting Hg species. The addition of an enzyme such as Protease XVI that destroys cell walls has proven to be successful in enhancing the release Hg from fish [11]. Mercury has been identified as binding to cysteine residues in proteins [12]; thus the use of an enzyme will rupture the proteins 3D structure allowing the chelating agent to access bound Hg species. However, it is also possible that Hg is present as nano-particles as reported for other plants that accumulate metals and metalloids [13].

Question 6: Do you understand the HPLC chromatography for separating Hg species

The most common HPLC techniques used to separate Hg species are based on the use of C18 (octyldecylsilane) or C8 (octylsilane) reverse-phase columns with polar mobile phases containing cysteine, mercaptoethanol or other S containing chelating agents [4] to prevent absorption of Hg species onto chromatographic components, enhance peak resolution and improve the shape of peaks. These chelates react with both Hg^+ and CH_3Hg^+ species to form near-neutral complexes, enabling the elution of Hg species from the column. The commonly used mobile phases contain different percentages of methanol or acetonitrile in deionised water containing the complexing agents. The pH of the mobile phase is then adjusted to pH 5-7 with a buffer solution (usually Na or NH_4 acetate) again to improve the separation of Hg species and peak shapes. No studies have given reasons for the choice of a specific mobile phase. The concentration of MeOH in the mobile phase is also important in obtaining good separation. MeOH concentrations higher than 5% (v/v) should be avoided as it can lead to ICPMS plasma instability and an increase in carbon residues on cones when ICPMS is used as a detector. Increasing the MeOH content of the mobile phase decreases the retention times of Hg species, however, the resolution of Hg^+ and CH_3Hg^+ species decreases when the MeOH concentration is higher than 5% (v/v).

Both types of columns give good separation of Hg species, however, the use of C8 columns reduces analysis times

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compared to the use of C18 columns due to less interaction of Hg species with the stationary phase. HPLC mobile phases should be selected based on the elution order of the expected Hg species (Figure 3A and B).

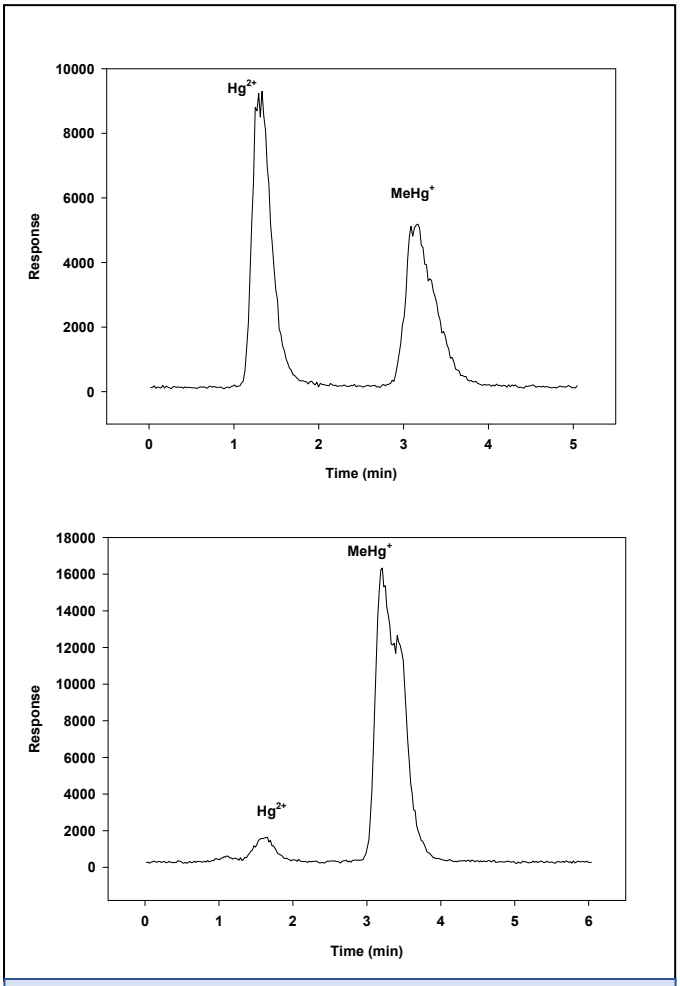

Figure 3: The elution profile of mixture of (A) Hg standards and (B) DORM-2 with mobile phase 5% methanol containing 0.06M ammonium acetate and 0.1% cysteine pH=6.8 [11].

In biota, CH_3Hg^+ concentrations will be much greater than Hg^{2+} concentrations, while in sediments the reverse occurs. To prevent swamping of the smaller Hg^{2+} species peak, it should be eluted first. As well, if Hg^{2+} concentrations in sediments are not required, the eluent containing this species can be directed to waste avoiding long wash out times and memory effects in the ICPMS. Optimising buffer strength, pH and temperature only improves the chromatography of the Hg^{2+}- S complex. The use of cysteine forms a Hg-cysteine complex that elutes after the CH_3Hg^+ species with mercaptoethanol forms a Hg^{2+} complex that is not retained on C8/C18 columns. We calculated the pKa's of mercaptoethanol complexes of $Hg^{2+}$and $CH_3Hg^+$to be 15.13 and 15.83 respectively and the pKa's of cysteine complexes of Hg^{2+} and CH_3Hg^+ to be 7.32 and 7.09, respectively. Thus' the mercaptoethanol complexes are charge neutral, while the cysteine complexes have a slightly positive charge at pH 5.3. It appears that the interaction of the CH_3Hg^+ complexes with the C8/C18 columns is via the methyl group as both the mercaptoethanol and cysteine complexes have the same retention time. The Hg^{2+} mercaptoethanol complex is charge neutral and strongly interacts with the C8C18 column (longer retention time), while the charged cysteine complex has less affinity for the column and has a much shorter retention time. Add the following sentence here.

Normally, direct coupling of HPLC to ICPMS is sufficient. If more sensitivity is required a flow injection hydride system can be used where the HPLC replaces the autosampler (Figure 1B).

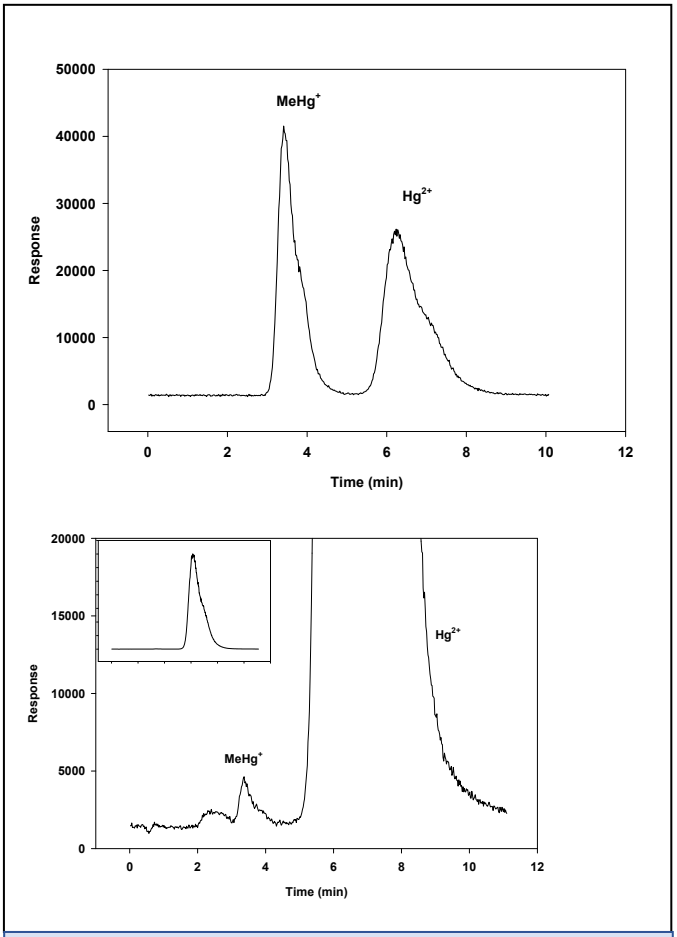

Figure 4: HPLC-ICPMS Chromatogram of (A) Hg standards and (B) sediment reference material, ERM CC 580 using A Perkin-Elmer 3 µm C8 (30 mm x 3 mm) and mobile phase containing 0.5% 2-mercaptoethanol and 5% v/v CH_3OH (pH 5.3) at a flow rate, 1.5 ml / min and a temperature of 25 °C. [10].

Question 7: Are you forming speciation artefacts

It has been widely reported that Hg species artefacts may be formed as the result of extraction procedures to remove Hg species from matrices. These reports, however, have been mainly when steam distillation is used to concentrate Hg species from sediments [14]. To our knowledge, no formation of artefacts has been reported when S-chelation is used to extract Hg species from biological and sediment matrices.

Question 8: What interferences is a problem in ICPMS measurements

Compared to other techniques such as AAS, AFS and ICPOES, ICPMS has emerged as the technique of choice for trace Hg analysis of environmental samples due to its excellent detection limits (0.001µg/L or lower), wide linear dynamic range and the ability to measure isotope ratios. Furthermore, because the Hg species can be measured directly in biological and sediments extracts, it is not necessary to reduce the Hg species to Hg^0 and, consequently, makes the procedure much simpler. Rai et al. [11] have reported that by adding a sulfur ligand such as cysteine to the mobile phase, Hg adsorption on HPLC–ICPMS components is reduced or eliminated. Thus, an added advantage of using a thiol-containing agent in the HPLC mobile phase is the reduction of Hg tailing during ICPMS measurements.

There are seven stable isotopes of mercury: ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, and ^{204}Hg and the ^{181}Ta and ^{184}W isotopes need to be measured to monitor the production of polyatomic oxides that can interfere with the quantification of mercury using these isotopes i.e., $^{184}W^{16}O$, $^{184}W^{17}O$, $^{184}W^{18}O$, $^{182}W^{16}O$, $^{182}W^{17}O$, $^{182}W^{18}O$, $^{186}W^{16}O$, $^{186}W^{17}O$, $^{186}W^{18}O$, $^{183}W^{16}O$, $^{183}W^{17}O$, $^{183}W^{18}O$, $^{181}Ta^{16}O$, $^{181}Ta^{17}O$, $^{181}Ta^{18}O$, $^{180}Ta^{16}O$, $^{180}Ta^{17}O$ and $^{180}Ta^{18}O$ [15].

Question 9: Should isotope dilution be used?

The use of ICPMS allows the use of isotope dilution to check species transformations and calculate extraction recoveries using enriched Hg isotopes [14]. Wilken and Falter [16] corrected for methylation artefacts produced during steam distillation by using an enriched stable Hg (II) isotope.

CONCLUDING REMARKS

Mercury analysis poses significant challenges mainly due to contamination issues and the low concentrations that need to be quantified. Clean up of acids is required while most other reagents can be purchased of sufficient purity for analysis purposes. The use of ICPMS gives the detection power to measure Hg in biota and sediments, but preconcentration or use of a hydride generation system is required to determine Hg in water samples. Hg speciation by HPLC-ICPMS is relatively easy as normally only two species, $Hg^{2+}$and CH_3Hg^+, need to be quantified and the use of cysteine or mecaptoehanol in the mobile phase prevent adsorption of Hg within the ICPMS.

Note: Papers cited not freely available can be obtained on request from authors

FUNDING

There is no funding sources

DISCLOSURE STATEMENT

The authors report there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

Data used in this paper is available in papers cited in references

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