Simultaneous combustion preparation for mercury isotope analysis and detection of total mercury using a direct mercury analyzer

Maxime Enrico [a, *], Prentiss Balcom [a], David T. Johnston [b], Julien Foriel [b], Elsie M. Sunderland [a, b]
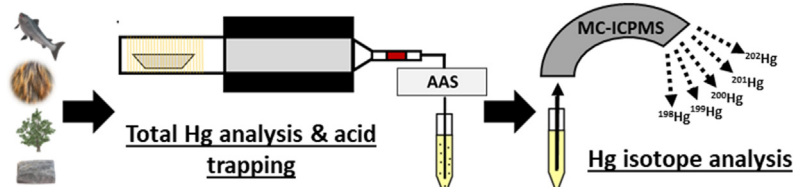
[a] Harvard John A. Paulson School of Engineering & Applied Sciences, Harvard University, 29 Oxford St, Cambridge, MA, 02138, USA
[b] Department of Earth and Planetary Sciences, Harvard University, 20 Oxford St, Cambridge, MA, 02138, USA

HIGHLIGHTS

- Preconcentration of Hg during total Hg analysis reduces preparation time and sample mass required.
- Sample preparation for Hg isotope analysis only takes ~8 min.
- Hg isotope analysis of SRMs matches literature data with good reproducibility.

GRAPHICAL ABSTRACT

ABSTRACT

Mercury (Hg) stable isotope signatures are widely used to understand Hg cycling in the environment. Sample preparation methods for determining Hg isotope ratios by CV-MC-ICP-MS vary widely among laboratory facilities and sample types. Here, we present a novel and rapid method for preparing solid samples prior to determining Hg isotope composition. We use a direct Hg analyzer (that measures total Hg) for sample combustion, amalgamation and analysis. During the thermal release of Hg from the amalgamator and following detection, the analyte gas enters a trapping solution consisting of 10% HCl/BrCl (5:1, vol/vol). We find Hg blank values are less than 1% of the Hg introduced during sample analysis, Hg detection is not altered by modifying the system, and more than 90% of the introduced Hg is recovered in the trapping solution. Hg isotope results are statistically indistinguishable from accepted values for previously published certified reference materials and uncertainty of 2σ (0.05−0.12‰) is similar to the solution standard RM8610 (2σ = 0.09‰). This new method allows for solid sample preparation for Hg isotope analysis in under 15 min. It has the additional advantage of minimizing use of sample mass during simultaneous detection and preparation.

1. Introduction

Mercury (Hg) stable isotopes are widely used to better understand Hg sources, sinks and transformations in the environment. Cold vapor − multicollector - inductively coupled plasma − mass spectrometry (CV-MC-ICP-MS) is most commonly used to measure Hg isotope ratios [1]. Applications of Hg isotopes include identifying Hg sources [2], tracing environmental redox biogeochemistry [3], and quantifying photochemical demethylation of methylmercury [4]. The Hg isotope system has characterized many different processes in diverse environmental samples, including atmospheric samples (gaseous Hg, rain and snowfall) [5,6], terrestrial materials (soils, rocks) [7,8], surface waters (lake and sea waters) [9,10], marine sediment [11], and aquatic biota [12,13]. The growing interest in Hg isotope research has led to analytical improvements

* Corresponding author.
E-mail address: menrico@seas.harvard.edu (M. Enrico).

[14,15] and new sample preparation methods [16,17]. Several methods are available for processing solid samples prior to Hg stable isotope analysis by MC-ICP-MS. All methods are constrained by the need for low acid concentrations (<20% vol) and sufficient Hg concentrations for precise Hg isotope ratio analyses (typically >0.5 ng mL^{-1}). Long Hg extraction procedures are needed to reach these specifications, preventing rapid processing of large sample batches.

Concentrations of Hg in aqueous samples (rain, snow, surface water and seawater) are generally lower than the range required for Hg isotope analysis. Therefore, samples are usually preconcentrated using a purge and trap method [18] or via an anionic exchange membrane [19]. For solid samples, Hg must first be dissolved into solution. Acid digestion is a convenient preparation method as many samples can be processed at once. However, some studies have shown matrix effects can alter the accuracy of ensuing isotope ratios [17]. Therefore, a purification step consisting of Hg reduction, purging from the digestion solution, and trapping in a clean oxidizing solution is recommended [17]. An intermediate step with purge and amalgamation on a gold trap, followed by heat desorption and acid trapping has also been shown to be efficient [20]. This method is not limited to solid samples, as it can also be used for atmospheric gaseous Hg (after sampling on a gold trap) and liquid samples.

Sample combustion is used by most other methods to prepare samples for Hg isotope analysis. Solid samples are introduced in a furnace and combusted under an O_2 headspace, with temperatures increasing from ambient to 800−1000 °C. All persistent Hg complexes in the gas are decomposed in a second tube furnace in series by heating at 1000 °C [21−23]. The combustion gases are then trapped in an oxidizing solution ($KMnO_4$ or inverse aqua regia). This entire combustion extraction process takes 3.5−7 h. Although time consuming, matrix effects are significantly reduced. Further, matrix cleaning by purge and trap might may improve the analysis, especially when using $KMnO_4$, which can precipitate over time. This combustion method, first developed at the University of Michigan [21], was adapted in different laboratories across the world, with notable variations in the trapping solution or in the temperature program. Fu et al. [24] added a catalyst from a total Hg analyzer in order to remove volatile halogen compounds. Given the time intensive nature of combustion methods, Zheng et al. [25] proposed a modification of a direct Hg analyzer (DMA, total Hg analysis) for preconcentrating Hg in acidic solutions. This approach also includes a catalyst filter for removing any potential interfering molecules (halogens, sulfur compounds, etc.). Instead of a few hours, this procedure requires only 20−30 min per sample and is quantitative with respect to recovery yield in the trapping solution.

The goal of the present study was to develop a robust, rapid procedure for sample concentration determination and preparation prior to Hg isotope ratio analyses. Combustion is preferred as it removes most of the sample matrix, which is especially important for samples with high organic content [17,21,22]. Many instruments dedicated to Hg analysis use combustion followed by catalyst filtration and amalgamation on gold beads, with combustion times of only a few minutes. Total Hg analysis is then performed by atomic absorption spectroscopy (AAS) detection following thermal decomposition of the gold-Hg amalgam within 30 s. Our approach consists of trapping the pulse of gaseous Hg produced during thermal release in a solution by sparging the analyte gas into an oxidizing solution. Trapping of Hg released from amalgams has been previously demonstrated [20]. Here we investigate the feasibility of an approach that significantly reduces sample preparation time by combining a robust combustion device, matrix removal by catalyst filtration, and Hg isolation by amalgamation prior to acid trapping. This approach enables evaluation of total Hg

concentrations during sample preparation by connecting the trapping solution downstream to the AAS detector.

2. Materials and methods

2.1. Reagents

There are three main reagents of interest within this method: 1) The trapping solution, 2) hydroxylamine hydrochloride ($NH_2OH.HCl$), and 3) stannous chloride (SnCl). The trapping solutions consisted of 10% HCl/BrCl (5:1, vol/vol). BrCl was prepared using EPA procedure 1631E [26] by dissolving KBr and $KBrO_3$ (J.T. Baker) in commercial HCl (J.T. Baker INSTRA ANALYZED reagent). Trapping solutions were prepared daily by diluting BrCl and HCl in deionized (DI) water. For BrCl neutralization before analysis, we used hydroxylamine hydrochloride ($NH_2OH.HCl$) prepared following EPA procedure 1631E [26] by dissolving $NH_2OH.HCl$ powder (J.T. Baker analyzed ACS reagent) in DI water. Finally, stannous chloride ($SnCl_2$) was used as a reductant for both cold vapor atomic fluorescence spectrometry (CVAFS) and during MC-ICPMS analysis. For CV-AFS analysis, a 10% $SnCl_2$ solution was prepared according to US EPA 1631E [26] by dissolving $SnCl_2.2H_2O$ (J.T. Baker analyzed ACS reagent) in 10% HCl. For MC-ICPMS analysis, we dissolved 30 g of $SnCl_2.2H_2O$ in 100 mL of HCl and diluted to 1L with DI water.

2.2. Reference materials

Two total Hg analyzers (Nippon Ma-3000 and Tekran 2600 automated Hg analyzer) were calibrated using dilute solutions prepared from a stock solution of an aqueous Hg standard (Inorganic Ventures, Hg concentration of 100.07 ± 0.54 μg mL^{-1} in 10% HNO_3, Lot P2-HG677723, hereafter noted as IV aqueous Hg CRM). Calibration curves were confirmed using diluted solutions from a secondary aqueous standard (SPEX CertiPrep, aqueous Hg concentration of 10 μg mL^{-1} in 5% HNO_3, Cat CLHG2-1AY, lot CL11-53HGY) for the Tekran aqueous Hg analyzer and with solid CRMs (see the CRMs below) for the MA-3000. The same IV aqueous calibration standard was also used for validating the combustion method.

For calibrating isotope analyses, we employed well known certified reference materials (CRMs). We purposefully included different matrices, such as pine needles (NIST 1575a), estuarine sediments (MESS-2), human hair (ERM-DB001), lobster hepato-pancreas (TORT-3) and fish protein (DORM-4). The reference material RM 8610 (formerly UM-Almaden) was analyzed for Hg isotope ratios every 10 analyses as a metric for analytical accuracy and precision.

2.3. Combustion system

We used a modified Nippon MA-3000 direct Hg analyzer (DMA, total Hg analysis) with an atomic absorption spectrometer (AAS, Fig. 1). The Hg analyzer was calibrated using dilute solutions of IV aqueous Hg CRM. Solid samples were combusted by heating at 175 °C for 2 min, then increasing the temperature to 850 °C over 1 min, and holding at this temperature for two more minutes. During combustion, a 0.4 L min^{-1} O_2 flow carried released gases through a catalyst filter to the amalgamator where the Hg was retained. Other gases passing through the amalgamator were carried to an outlet without passing through the detection cells. When sample combustion was complete, the amalgamator was heated to >600 °C in 30 s to release the Hg to the detection cells under a 0.2 L min^{-1} O_2 flow. Approximately 10 s prior to the heating step, the trapping solution was manually installed at the outlet of the
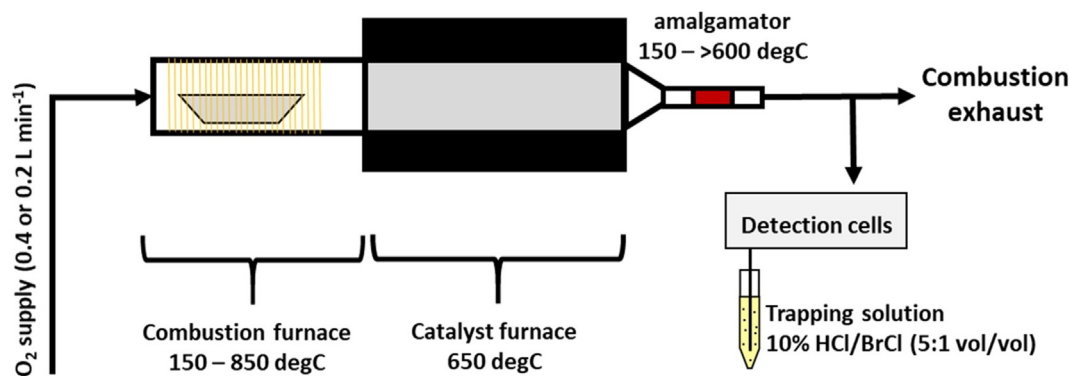
Fig. 1. Schematic of the direct Hg analyzer (DMA) combustion system used in this study. A valve system is used at the outlet of the amalgamator to direct released gases either to exhaust (during combustion) or to the AAS detection cells (during heat desorption).

detection cells by connecting a 1/8 inch Teflon tube from the detection cell to the trapping solution. The outlet of the tube was restricted to 0.02 inch (internal diameter, Teflon tubing) to produce smaller bubbles with a high surface area to volume ratio in the solution. The trapping solution was removed 10 s after the end of the amalgamator heating stage, so that the bubbling step only took ~50 s. The outlet of the detection cell was then reconnected to the usual circuit for the next sample.

2.4. Hg concentrations in trapping solutions

After the combustion-trapping procedure, total Hg concentrations were measured in solution using a Tekran 2600 CVAFS automated Hg analyzer following Hg reduction, purge and trap, and thermal release according to US EPA EPA [26]. A 0.2 mL aliquot of trapping solutions was diluted to ~30 mL of DI water with 0.1 mL of BrCl in 40 mL glass vials with Teflon-faced septum caps. Prior to analysis, excess BrCl was neutralized by the addition of 0.05 mL of 12% $NH_2OH.HCl$, and 0.05 mL of 50% $SnCl_2$ was added for Hg reduction. For blanks (solutions, boat blanks and DI blanks), a larger aliquot (5 mL) was used to better quantify Hg concentrations.

2.5. Hg isotope analysis

The determination of Hg stable isotope ratios was performed using a Thermo Neptune MC-ICP-MS plus in the Johnston laboratory at Harvard University. The CRM trapping solutions were analyzed for Hg isotope ratio without further dilution. Prior to analysis, the excess BrCl was neutralized by the addition of 0.1 mL of hydroxylamine hydrochloride. The sample solution was pumped and mixed with 3% $SnCl_2$ (in 10% HCl) at ~0.6 mL min^{-1} in a Teledyne Cetac HGX-200 hydride generator. The reduced and volatilized Hg in the HGX-200 was transported to the plasma by sample and additional gases (Argon). We used an Apex-Q (Elemental Scientific) for nebulizing a Thallium solution and injecting Tl aerosols in the HGX-200. A dilute (50 ppb in 2% HNO_3) solution of NIST997 (recommended $^{205}Tl/^{203}Tl = 2.38714$) was introduced in the Apex-Q at 50 μL min^{-1} using a micronebulizer. The aerosols were produced and injected to the HGX-200 with the additional gas. The set-up of the HGX-200 was similar to Geng et al. [15].

We detected five Hg isotopes (^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg) and two Tl isotopes (^{203}Tl and ^{205}Tl). The Hg isotope ratios were corrected for mass bias by applying an exponential correction based on the known $^{205}Tl/^{203}Tl$ ratio of 2.38714 for NIST997. We adopted common standard-sample bracketing by pairing unknowns with a dilute solution of NIST3133 (prepared at similar concentrations and in a matrix similar to sample solutions). We

report Hg isotope signatures as common delta values [27]:

$$\delta^{xxx}Hg = \left(\frac{\left(^{xxx}Hg/^{198}Hg \right)_{sample}}{\left(^{xxx}Hg/^{198}Hg \right)_{NIST3133}} - 1 \right) \times 1000 \quad , .and$$

$$\Delta^{yyy}Hg = \delta^{yyy}Hg - \beta_{yyy} \times \delta^{202}Hg$$

where ^{xxx}Hg represents ^{199}Hg, ^{200}Hg, ^{201}Hg or ^{202}Hg, and ^{yyy}Hg reflecting ^{199}Hg, ^{200}Hg, or ^{201}Hg. β_{yyy} is the mass-dependent correction factor between $\delta^{xxx}Hg$ and $\delta^{202}Hg$ (0.2520, 0.5024 and 0.7520 for β_{199}, β_{200} and β_{201}, respectively).

3. Results and discussion

3.1. Blank values

In developing this method, we quantified both solution and procedural blanks. The average daily Hg concentration in the trapping solution was 0.2 ± 0.1 pg mL^{-1} (1σ, n = 4, Table 1), 5000-times lower than the target concentration for samples (~1 ng mL^{-1}) and thus negligible for our results (2 pg of Hg in 10 mL of trapping solution). Different procedural blanks were also assessed during the analyses. The DMA system was first cleaned by running empty boats, and boats loaded with 100 μL of DI water. Then, the sequence started with 100 μL of DI water (DI samples, 240 s at 850 °C) and a blank with an empty boat (boat blank, using a combustion method similar to samples). The initial DI DMA results showed a background Hg release of 24 ± 24 pg (1σ, n = 4) according to the DMA calibration (Table 1). The CV-AFS analysis of solutions reveal a statistically indistinguishable DI blank concentration (release) of 33 ± 16 pg of Hg (1σ, n = 4). Any difference between the two

Table 1
Summary of blank Hg concentrations.

Blank type[c]	n	DMA result (pg Hg)	Solution (pg Hg)
solution blanks[a]	4		2 ± 1
initial boat blanks[b]	4	4 ± 6	22 ± 11
procedural boat blanks	12	39 ± 23	91 ± 30
initial DI blanks[b]	4	24 ± 24	33 ± 16
procedural DI blanks	12	163 ± 102	183 ± 78

[a] Unprocessed solution blanks.
[b] First of day after cleaning analyses.
[c] Results are presented as pg of Hg measured by the AAS direct Hg analyzer (DMA) and as determined from CV-AFS analysis of the trapping solutions.

evaluations is likely related to the DMA calibration, which corrects for background Hg release. The DI solution value of 33 pg is an order of magnitude higher than the trapping solution blank, but still represents a low signal to noise ratio on the order of 500. The Hg released during the initial boat blanks is slightly lower but within error of that noted above (22 ± 11 pg, 1σ, n = 4, Table 1). Any difference could reflect a cleaner system due to the previous DI blank, and/or more efficient desorption of Hg from the system by water vapor.

During our analyses, a procedural boat blank was measured every five samples to evaluate the possibility of Hg memory effects. For a more efficient cleaning, this boat blank sample was followed by an analytical system purge with 100 μL of DI water. In both cases, a trapping solution was connected to evaluate the procedural blanks. After the DI cleaning, another purge (cleaning) analysis (no DI) was performed prior to preparing the next sample. The procedural boat blanks averaged 91 ± 30 pg, which was higher than the initial boat blanks (Table 1) but still represented only ~1% of the Hg introduced during sample introduction. The procedural DI blanks (purges) released twice as much Hg (183 ± 78 pg). None of the blank values were critical as they never exceeded 2% of the Hg introduced during sample processing. The efficient release of Hg during the DI purges helped maintain a clean instrument, and this procedure might therefore be necessary to avoid significant Hg carryover.

3.2. Hg recovery yield

It is necessary to report the recovery of Hg in solution to evaluate the efficiency of any digestion, combustion or preconcentration method. We used calibrated CRMs to evaluate Hg extraction yields. We monitored Hg concentrations (DMA) and trapping solution (preconcentrate) Hg from the same sample aliquot. Our daily procedure included a leak check prior to warming up the different parts of the Hg analyzer. All paths (drying exhaust, combustion exhaust and analysis exhaust) were tested to confirm the absence of leaks, and to ensure no Hg was lost during the process. On several occasions during preliminary tests, CRMs were prepared while the leak check indicated minor losses and led to Hg recovery yields lower than 65%. This usually does not affect Hg concentration measurements if the analyzer was calibrated under similar conditions, but could potentially lead to erroneous Hg isotope ratios (not measured in this study) due to mass-dependent fractionation. All results reported here were obtained under optimal conditions (no leak), an important pre-requisite for ensuring high recovery yields.

Hg recoveries were calculated relative to either the certified concentration or the result of the total Hg analysis. Total Hg concentrations measured with the DMA agreed with certified values for all CRMs tested, with relative standard deviations (RSD) of less than 5% (Fig. 2, Table S1). The highest RSD (5%) was found for the hair CRM ERM-DB001, while others were 2% or less. Average values determined by the DMA were sometimes lower than the certified values (e.g., ERM-DB001, DORM-4, see Table S1 and Fig. 2) but mostly equivalent to them within their associated uncertainties; however we cannot exclude minor losses during DMA analysis (Fig. 2, Table S1). We also analyzed the Hg in trapping solutions (CV-AFS) and evaluated the recovery based on the certified concentration and the amount of Hg detected by DMA during analysis/preconcentration. The average overall recovery based on certified values was 90 ± 4%. Matrix specific recoveries varied between 87 ± 3% (Human hair ERM-DB001, 1σ, n = 10) and 94 ± 3% (IV aqueous Hg CRM, 1σ, n = 10). These values compare the average certified Hg concentrations in CRMs to those we recovered in solution. Therefore, this comparison does not take into account the actual Hg concentration in our CRM batches.

We also calculated Hg recoveries as the ratio of solution Hg (determined by CV-AFS) to Hg concentration measured during the simultaneous AAS analysis. Simultaneous and independent analysis of Hg and acid trapping allows for an evaluation of Hg recovery from the same sample aliquot. We found an average Hg recovery of 92 ± 3%. The lowest values were for pine needles and highest values for IV aqueous Hg CRM (90 ± 4% and 95 ± 3%, respectively, Table S1 and Fig. 2). Although generally similar, the calculated Hg recovery significantly increased for human hair and fish protein CRMs (from 87 ± 2% to 94 ± 3%, and 87 ± 2% to 93 ± 2%, respectively, see Fig. 2 and supporting Table S1), due to the slightly lower measured total Hg concentrations compared to the certified average values.

The processing of pine needles (CRM 1575a) slightly differed from the other CRMs. The relatively low Hg concentration (39.9 ± 0.7 ng g^{-1}) and low density of this material made loading sufficient mass into one single sample boat impractical. Pine needles (approximately 250 mg) were therefore split into two sample boats. The two sample aliquots were successively combusted and preconcentrated in the amalgamator prior to release and detection. This procedure did not affect Hg concentration analysis (39.5 ± 0.8 ng g^{-1} for a certified Hg concentration of 39.9 ± 0.7 ng g^{-1}) and also resulted in a high Hg recovery yield in the trapping solution (90 ± 4%). The demonstrated feasibility of preconcentrating Hg from multiple aliquots indicates the reliability of this method for processing samples with low Hg concentration (e.g. bedrock) or with low density (e.g., foliage, litter, soil).

3.3. Mercury isotope in aqueous solutions

Analytical uncertainty in Hg isotope measurements is commonly evaluated using the RM8610 secondary standard. Results of our repeated analyses of RM8610 were in perfect agreement with reference values (δ^{202}Hg values of −0.55 ± 0.09‰, Δ^{199}Hg of −0.02 ± 0.08‰, Δ^{200}Hg of −0.01 ± 0.07‰ and Δ^{201}Hg of −0.04 ± 0.07‰, 2σ, n = 18, Table 2). Because RM8610 solutions were prepared by dilution, this analytical uncertainty does not include the variability potentially induced by the combustion procedure and associated minor matrix differences. In addition, RM8610 and NIST3133 (bracketing standard) were diluted in 10% HCl (no BrCl) and were not prepared by introducing oxygen gas in the solution.

To investigate the potential for a solution matrix effect due to BrCl or oxygen bubbling (i.e., DMA analysis), the blank solutions (not DI blanks) were spiked with RM8610 and analyzed together with samples. We found average δ^{202}Hg values of −0.57 ± 0.10‰, Δ^{199}Hg of 0.03 ± 0.06‰, Δ^{200}Hg of 0.00 ± 0.05‰ and Δ^{201}Hg of −0.03 ± 0.07‰ (2σ, n = 8). This matched the RM8610 values found by diluting in non-processed solutions (Table 2), meaning that the DMA oxygen flow caused no substantial matrix effect.

Similar to RM8610, we evaluated the Hg isotope composition of diluted solutions of IV aqueous Hg CRM. We measured an Hg isotope composition relatively close to RM8610, with δ^{202}Hg of −0.66 ± 0.10‰, Δ^{199}Hg of 0.02 ± 0.10‰, Δ^{200}Hg of 0.01 ± 0.08‰ and Δ^{201}Hg of −0.01 ± 0.06‰ (2σ, n = 10). The same IV aqueous Hg CRM prepared by the combustion method resulted in slightly lower δ^{202}Hg of −0.73 ± 0.05‰ (average ± 2σ, n = 10, t-test p < 0.01) and similar Δ^{200}Hg of 0.04 ± 0.06‰ (2σ, n = 10, t-test p > 0.01). Although there is overlap within 2σ uncertainty, the comparison of the means with 2 standard errors (2SE) indicates a slight difference for δ^{202}Hg (−0.66 ± 0.03‰ vs −0.73 ± 0.02‰, both 2SE, n = 10). Similarly, odd Hg isotope anomalies displayed significantly positive though variable signatures with averages of 0.18 ± 0.12‰ (2SE = 0.04‰) and 0.12 ± 0.16‰ (2SE = 0.05‰) for Δ^{199}Hg and Δ^{201}Hg respectively (both t-test p < 0.01).
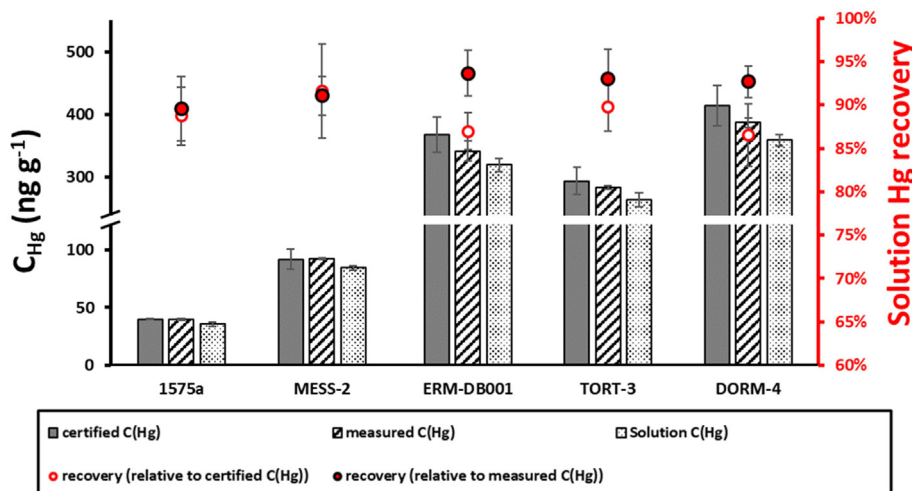
Fig. 2. Comparison of Hg concentrations (bars) found in the certified reference materials (CRMs) using the direct Hg analyzer with AAS detection (measured C(Hg)), and from Hg trapped in solution (Solution C(Hg)) using the CVAFS analyzer, with certified Hg concentrations (certified C(Hg)). Circles denote the solution Hg extraction yield calculated based on certified concentrations (open red circles) and AAS detection results (red filled circles). Note the axis break for Hg concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Hg isotope results obtained for the CRMs evaluated in this study compared with other reported values.

CRM	Matrix	reference	n	δ^{202}Hg	Δ^{199}Hg	Δ^{200}Hg	Δ^{201}Hg
				‰ (± 2σ)	‰ (± 2σ)	‰ (± 2σ)	‰ (± 2σ)
RM8610	dilution	This study	18	−0.55 ± 0.09	−0.02 ± 0.08	−0.01 ± 0.07	−0.04 ± 0.07
	blank spiked	This study	8	−0.57 ± 0.10	0.03 ± 0.06	0.00 ± 0.05	−0.03 ± 0.07
	—	Reference value[a]		−0.56 ± 0.03[a]	−0.03 ± 0.02[a]	0.00 ± 0.01[a]	−0.04 ± 0.01[a]
IV aqueous Hg CRM	dilution	This study	10	−0.66 ± 0.10	0.02 ± 0.10	0.01 ± 0.08	−0.01 ± 0.06
	combustion	This study	10	−0.73 ± 0.05	0.18 ± 0.12	0.04 ± 0.06	0.12 ± 0.16
1575a	Pine needles	This study	10	−1.30 ± 0.10	−0.35 ± 0.08	0.02 ± 0.06	−0.37 ± 0.10
		[17]	7	−1.32 ± 0.05	−0.37 ± 0.11	0.01 ± 0.05	−0.30 ± 0.08
MESS-2	Estuarine sediment	This study	10	−1.73 ± 0.05	0.02 ± 0.07	0.01 ± 0.04	−0.03 ± 0.09
		[33]	3	−1.84 ± 0.11	0.03 ± 0.03	NR	NR
		[34]	9	−1.93 ± 0.10	−0.02 ± 0.04	0.03 ± 0.04	−0.04 ± 0.05
		[36]	3	−2.10 ± 0.10	0.04 ± 0.05	0.01 ± 0.02	−0.01 ± 0.03
		[35]	3	−1.95 ± 0.03	0.01 ± 0.04	0.05 ± 0.04	NR
ERM-DB001	Human hair	This study	7	1.98 ± 0.12	1.30 ± 0.14	0.04 ± 0.11	1.01 ± 0.12
TORT-3	Lobster hepatopancreas	This study	10	0.06 ± 0.06	0.72 ± 0.07	0.07 ± 0.06	0.59 ± 0.11
		[17]	4	0.07 ± 0.08	0.67 ± 0.10	0.06 ± 0.12	0.55 ± 0.16
		[13]	7	0.13 ± 0.12	0.69 ± 0.10	NR	NR
		[39]	4	0.05 ± 0.08	0.66 ± 0.04	NR	NR
DORM-4	Fish protein	This study	10	0.45 ± 0.08	1.83 ± 0.06	0.07 ± 0.08	1.47 ± 0.12
		[17]	10	0.47 ± 0.09	1.81 ± 0.09	0.07 ± 0.06	1.48 ± 0.06
		[13]	7	0.47 ± 0.14	1.74 ± 0.18	NR	NR

NR = not reported.
   [a] Only RM8610 has reference values for Hg stable isotope composition, other values are reported from different studies. Uncertainties are 2 standard errors on results obtained by five different laboratories (number of analyses not reported).

The DMA combustion procedure for aqueous solutions included a drying step. For the MA-3000, this consisted of a 60 s heat step to 150 °C when volatilized compounds were not carried through the catalyst and amalgamator and went directly to exhaust, allowing for some minor losses of Hg. The small difference in δ^{202}Hg indicates small MDF associated to these losses, while the positive odd isotope anomalies suggest MIF occurred. Large MIF anomalies are usually attributed to photochemical reactions [4,28], which is unlikely to occur in the combustion component of the Hg analyzer. Smaller magnitude MIF can occur through nuclear volume fractionation. The small magnitude MIF and the higher Δ^{199}Hg compared to Δ^{201}Hg supports nuclear volume effect as a potential cause of these anomalous results.

This result contrasts with previous evaluations of NVE fractionation. Larger shifts in δ^{202}Hg compared to Δ^{199}Hg were reported in prior work [29–31]. We observed only a small difference in δ^{202}Hg. In addition, our results suggest that Hg volatilized during the drying step (Hg^0 vapor) had negative Δ^{199}Hg and Δ^{201}Hg, which is opposite the fractionation observed during liquid-vapor equilibrium [30] and to predictions of MIF during NVE at room temperature [32]. While this shift in odd isotope anomalies remains unclear, this effect was not observed for solid materials. The combustion program for solids does not include the drying step and all combustion gases are carried through the amalgamator.

3.4. Hg isotope composition of sediments, hair and biomass CRMs

Precision (2σ uncertainty) for isotope analyses of the different CRMs is in-line with that of RM8610 on all Hg isotope signatures (δ^{202}Hg, Δ^{199}Hg, Δ^{200}Hg and Δ^{201}Hg, see Table 2). Solid CRMs are

not certified for Hg stable isotope signatures. Method validation however requires some comparison, so that the Hg isotope composition of several CRMs can be found in the literature, providing some traceability. The accuracy of our results agrees within error bounds of the published value for these CRMs (Fig. 3). The pine needle CRM (NIST1575a) prepared by successive combustion of two sample boats by DMA had an Hg isotope composition consistent with previously reported values (Fig. 3 and Table 2), demonstrating that sample boat size constraints can be overcome by performing and then merging multiple combustions. Samples with similar matrices, such as leaf litter and various soils are amenable to this method.

Our results for estuarine sediment MESS-2 indicate an average δ^{202}Hg of -1.73 ± 0.05‰ (2σ, n = 10, Table 2). Previously reported δ^{202}Hg signatures for this CRM are significantly lower with averages of -1.84 ± 0.11‰ (2σ, n = 3, acid digestion) [33], -1.93 ± 0.10‰ (2σ, n = 9, combustion) [34], -1.95 ± 0.03‰ (2σ, n = 3, acid digestion) [35] and -2.10 ± 0.10‰ (2σ, n = 3, acid digestion) [36] (Fig. 3). The δ^{202}Hg result on MESS-2 is the only Hg isotope value we report that does not agree with previously reported values (Fig. 3). This can be attributed to variability between batches (CRM certified for Hg concentration only), as was also observed for MESS-3 [17], the successor to MESS-2. Despite this variability in δ^{202}Hg, the Δ^{199}Hg we report (0.02 ± 0.07‰, 2σ, n = 10) is consistent with previous analyses within 2σ.

The human hair CRM (ERM-DB001) displayed a δ^{202}Hg of 1.98 ± 0.12‰ (2σ, n = 7). Among our analyses, this was the highest average δ^{202}Hg, which relates to the mass-dependent fractionation commonly observed between consumed fish (the main Hg exposure pathway) and hair [12]. The Δ^{199}Hg (1.30 ± 0.14‰, 2σ, n = 7) was also elevated and similar to fish. For both δ^{202}Hg and Δ^{199}Hg, the 2σ uncertainties we report are the highest among our analyzed CRMs. This could reflect slightly more heterogeneity in this reference material, as indicated by the higher RSD during total Hg analyses (5%, Table S1). This may have been exacerbated by our use of very small masses owing to the relatively high Hg concentration in this CRM. Nevertheless, the 2σ of 0.12‰ and 0.14‰ on δ^{202}Hg and Δ^{199}Hg respectively are still acceptable and well below the natural variations in δ^{202}Hg observed in human populations [12,37,38]. Unfortunately, no prior study has reported Hg isotope values for this CRM and thus comparison is not possible.

Average values for δ^{202}Hg and Δ^{199}Hg in lobster CRM (TORT-3) were 0.06 ± 0.06‰ and 0.72 ± 0.07‰ respectively (both 2σ, n = 10). These results are undistinguishable from other reported values within the 2σ range [13,17,39]. Similarly, results obtained for the fish CRM DORM-4 displayed δ^{202}Hg of 0.45 ± 0.08‰ and Δ^{199}Hg of 1.83 ± 0.06‰ (2σ, n = 10) and compare well with other studies (Fig. 3, Table 2) [13,17]. The low 2σ ranges (<0.10‰ for both δ^{202}Hg and Δ^{199}Hg) suggests the method performs well for marine biomass, with precision comparable to data generated through the long combustion procedure (Table 2) [17]. Other reported values for CRMs prepared by acid digestion showed both higher [13] and similar [39] 2σ on replicate analyses of TORT-3 and DORM-4 (Fig. 3, Table 2).

All Hg isotope signatures (δ^{202}Hg, Δ^{199}Hg, Δ^{200}Hg and Δ^{201}Hg) are comparable with other reported values for the CRMs used in this study, with relatively low 2σ uncertainties. Evaluation of the results for Δ^{200}Hg is complicated by the small variations among CRMs. Even Hg isotope anomalies are observable in atmospheric samples, with positive Δ^{200}Hg in oxidized Hg (rainfall) and slightly negative Δ^{200}Hg in gaseous Hg^0 [5,6,40,41]. The difference between atmospheric Hg species is however relatively low (below 0.3‰), only slightly above the common analytical 2σ. Other environmental samples have intermediate Δ^{200}Hg, so that their Δ^{200}Hg signature overlap within 2σ uncertainty. To our knowledge, no existing CRM exhibits a Δ^{200}Hg anomaly that is distinguishable from others within a 2σ range.

The Δ^{201}Hg values we report for the different CRMs are consistent with other studies as well (Table 2). We note that for pine needles, estuarine sediments and aqueous Hg standards, the Δ^{201}Hg is undistinguishable from Δ^{199}Hg. For hair, lobster and fish tissues, the Δ^{201}Hg is significantly lower than Δ^{199}Hg, with Δ^{199}Hg/Δ^{201}Hg ratios in between 1.23 and 1.28. MIF of odd Hg isotopes during photochemical reactions might be expressed differently according to the Hg species affected. In particular, photoreduction of inorganic Hg produces slopes ~1 [4,42−44] while photodegradation of methylmercury produces slopes ~1.3 [4] with larger fractionation factors. Aquatic biota, as well as human hair, accumulate methylmercury from dietary uptake, thus the larger Δ^{199}Hg and Δ^{201}Hg and the Δ^{199}Hg/Δ^{201}Hg ratio reflects the differences in the Hg species accumulated.

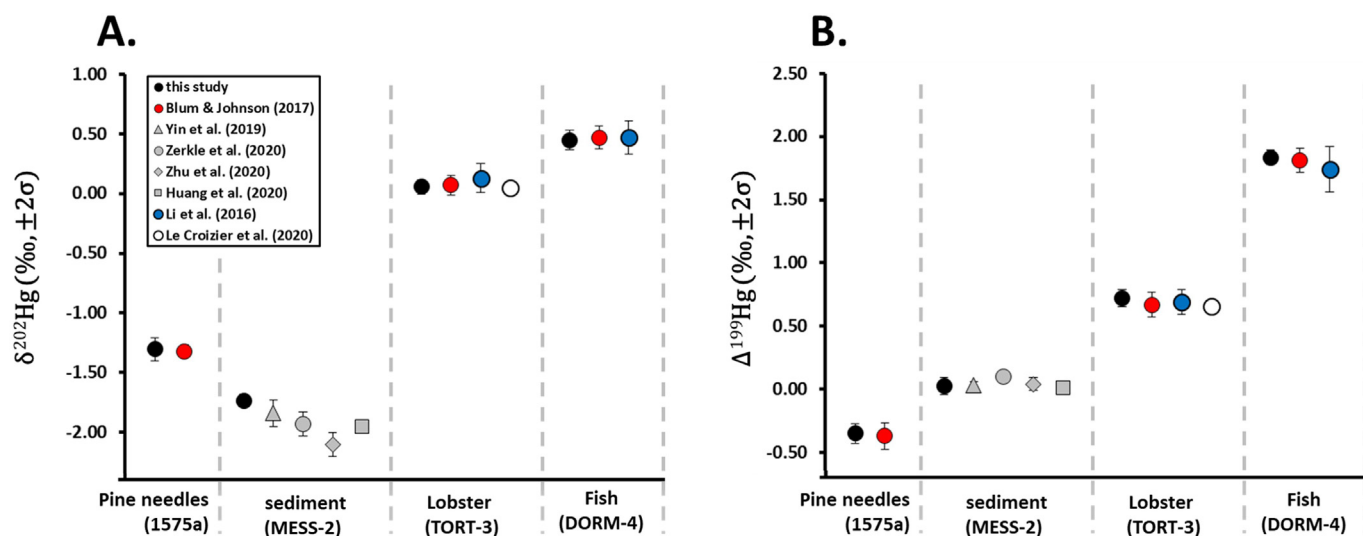**Fig. 3.** Comparison of the results of (A) δ^{202}Hg and (B) Δ^{199}Hg obtained for the analysis of reference materials (CRMs) in this study with values reported elsewhere [13,17,33−36,39]. When 2SE was reported instead of 2σ in the literature, 2σ was recalculated from the 2SE and the number of analyses. Blum and Johnson [17] and Zerkle et al. [34] prepared the CRMs with a long combustion procedure, and Li et al. [13], Yin et al. [33], Huang et al. [35], Zhu et al. [36], and Le Croizier et al. [39] acid digested the CRMs.

3.5. Advantages of the new combustion preparation method

Hg isotope analysis requires high recoveries in order to limit fractionation during the procedure. With a long combustion procedure, Sun et al. [22] did not observe any significant difference in Hg isotope signatures with Hg recoveries in the range 85−110%, yet their 2σ uncertainties for δ^{202}Hg of 0.18 and 0.22‰ suggest some mass-dependent fractionation. Our method differs from Sun et al. [22] by including an amalgamation step and releasing Hg as a pulse. The possibility of Hg isotope fractionation might therefore be more critical in the case of low recovery due to incomplete release from the amalgamator or inefficient oxidation in the solution. The evaluation of Hg recovery is therefore important here, and possibly in longer combustion procedures as well. For environmental samples, total Hg concentrations must be determined before isotope analysis to evaluate recovery, while extraction yields from CRMs are evaluated using certified concentrations. This may result in some variability related to sample heterogeneity. The method presented here determines Hg concentrations and preconcentrates Hg simultaneously from the same aliquot. The influence of sample heterogeneity on calculated Hg recovery is therefore negligible. Since Hg isotope analysis generally requires two aliquots (total Hg analysis and Hg extraction for Hg isotope measurement), the simultaneous detection and preconcentration with the current procedure is also of interest when limited sample mass is available, opening up a whole new set of environmental samples and questions. The final solution volume (10 mL) is sufficient for one Hg isotope analysis, but perhaps too small for duplicate Hg isotope measurements. Repeating the entire procedure, a true measure of reproducibility, provides redundancy for both Hg concentration and Hg isotope ratios.

Hg extraction methods developed over the last two decades are based on either sample digestion or combustion, which have differential matrix effects. For example, digestion may produce matrix effects that affect Hg isotope analysis [17] and, therefore, may require additional purification steps prior to isotope analysis. In comparing results for the CRMs tested in this study, we did not find strong evidence for any preparation method-related differences. This contrasts with observations reported by Blum and Johnson [17], but is consistent with Janssen et al. [20] who did not find any difference between direct analysis of digested samples and analysis after matrix purification. Matrix effects might arise for samples with low Hg concentrations because of the need of digesting large sample masses in small acid volumes. Combustion methods are more practical because the sample matrix is decomposed during combustion, limiting the retention of matrix material in the trapping solution. With the current method, the sample is combusted and released gases (Hg + part of the matrix) are carried through a catalyst filter that further purifies the gas. The Hg is then trapped on an amalgamator, and the potential residual matrix passes through and is directed to the outlet. In the DMA, a by-pass drives the gases transported through the catalyst and amalgamator during combustion to the outlet without passing through the detection cells. In such a system, the sample matrix that reaches the trapping solution is therefore limited to compounds that are trapped in the amalgamator and released during the heating step. Thanks to the amalgamation step, the method can be applied to any solid sample, with no difference in Hg recovery due to the sample matrix (litter, sediments, hair, biota). Although Blum and Johnson [17] recommended a purification step after Hg extraction by combustion, we do not find this is necessary as we find isotope signatures for CRMs comparable to results obtained with other preparation methods with low 2σ uncertainties, comparable to diluted RM8610.

A disadvantage of traditional combustion methods is that they are slow, due mainly to the gradual temperature ramping rate [21−23]. The method presented here allows the measurement and preparation of 6−7 samples per hour using only 10 ng of Hg, a significant increase in sample throughput capacity. Further improvement of MC-ICP-MS sensitivity [15] could push the sample mass thresholds even lower. The possibility of preparing large numbers of samples is an important advantage of digestion over combustion methods. Our method can be applied to only one sample at a time (except if more than one Hg analyzer is available). Combustion following our procedure is fast (~5 min) and the total time, including analysis and cooling, never exceeds 8 min per sample. The amalgamator heating step releases all Hg within a few seconds, and the trapping of such pulse of Hg requires a strong oxidizing solution. The 10% HCl/BrCl (5:1, vol/vol) solution showed good recoveries despite the relatively low acid concentration.

The solution can be adapted, as we noted similar recoveries with 5% HCl/BrCl (5:1, vol/vol, not shown) in preliminary evaluations. We chose a 10% acid mixture because it can be analyzed without further dilution and is sufficiently stable for long-term storage. However, we do not recommend the use of a 40% HNO_3/HCl (2:1, vol/vol) used in other long combustion procedures [22,23], because recoveries were only in the range 40−60%. As previously observed, a 40% HNO_3/HCl mixture efficiently traps Hg during long combustion procedures but is not capable of efficiently trapping a pulse of Hg from a gold trap [20].

The use of a modified total Hg analyzer is also an advantage by itself, as such instrument is already present in most laboratories that conduct Hg stable isotope analysis. We found the Nippon MA-3000 total Hg analyzer (direct Hg analyzer or DMA) was convenient as the outlet of the detector and was easily accessible and allowed for a short path from the outlet of the detector to our trapping solution (~25 cm), thus avoiding significant adsorption issues. In addition, the detector by-pass during combustion might have reduced the transfer of potentially interfering sample matrix to the trapping solution.

4. Conclusion

We developed a rapid and quantitative method for simultaneous Hg concentration analysis and preparation for Hg isotope measurements that is accessible to most laboratories conducting research on Hg in the environment. The method produces precise and accurate Hg concentrations and isotope measurements, and high recovery yields. This suggests that Hg is efficiently transferred from the sample to the amalgamator, and from the amalgamator to the detector and outlet. Obtaining minimal losses during the process is important for avoiding Hg isotope fractionation. Our Hg isotope data match accepted values, suggesting insignificant isotope fractionation occurs during our procedure as soon as the system is free of leaks.

CRediT authorship contribution statement

Maxime Enrico: Conceptualization, Methodology, Validation, Investigation, Writing − original draft. **Prentiss Balcom:** Resources, Writing - review & editing. **David T. Johnston:** Resources, Writing - review & editing, Funding acquisition. **Julien Foriel:** Resources, Writing - review & editing. **Elsie M. Sunderland:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

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