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Mercury speciation and total trace element determination of low biomass biological samples

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Abstract

Current approaches to mercury speciation and total trace element analysis require separate extraction/digestions of the sample. Ecologically important aquatic organisms, notably primary consumers such as zooplankton, polychaete and amphipods, usually yield very low biomass for analysis even with significant compositing of multiple organisms. Individual organisms in the lower aquatic food chains (mussels, snails, oysters, silversides, killifish) can also have very low sample mass, and analysis of whole single organisms is important to metal uptake studies. A method for the determination of both methyl Hg and total heavy metal concentrations (Zn, As, Se, Cd, Hg, Pb) in a single, low mass sample of aquatic organisms was developed. Samples (2 to 50 mg) were spiked with enriched with ²⁰¹MeHg and ¹⁹⁹Hg, then leached in 4M HNO₃ at 55 °C for extraction of MeHg. After 16hrs an aliquot (0.05 mL) was removed for determination of mercury species (methyl and inorganic Hg) by isotope dilution gas chromatography inductively coupled plasma mass spectrometry (ICP-MS). The leachate was then acidified to 9M HNO₃ and digested in a microwave at 150 °C for 10 minutes, and total metal concentrations were determined by collision cell ICP-MS. The method was validated by analysis of 5 biological certified reference materials. Average percent recoveries for Zn, As, Se, Cd, MeHg, Hg_{total}, Pb were 99.9%, 103.5%, 100.4%, 103.3 %, 101%, 97.7%, and 97.1 % respectively. The correlation between the sum of MeHg and inorganic Hg from the speciation analysis and total Hg by conventional digestion of the sample was determined for a large sample set of aquatic invertebrates (n=285). Excellent agreement between the two measured values was achieved. This method is advantageous in situations where sample size is limited, and where correlations between Hg species and other metals are required in the same sample. The method also provides further validation of speciation data, by corroboration of the sum of the Hg species concentrations with the total Hg concentration.

Keywords

Mercury speciation; trace metals; species-specific isotope dilution; low mass samples; inductively coupled plasma mass spectrometry; acid leaching; microwave digestion

Introduction

Environmental and human health concerns related to the accumulation of heavy metals have created a strong interest in studying the transfer of heavy metals through aquatic ecosystems [1,2, 3]. Such studies often require determination of both total metals (Zn, As, Se, Cd, Hg, Pb) and metal species in the same sample. In particular, Hg biomagnifies in aquatic ecosystems mostly as CH₃-Hg (MeHg), and determination of Hg species is crucial to toxicity and uptake studies. Determination of total metals and Hg species are usually performed by two separate analyses, as extraction of MeHg from aquatic organisms requires a low temperature leach, so as not to break the CH₃-Hg bond, whereas total metal determination in tissue requires complete dissolution of the sample matrix. Due to

limitations of sample collection and individual sample size, determination of both Hg species and total metal concentrations in the same sample is often advantageous. Metal uptake in aquatic organisms within the same population often varies with size and age, particularly when diet, and therefore trophic level, of an organism changes throughout their lifecycle [4]. It is therefore valuable in ecosystem studies to analyze individual whole organisms, rather than composite samples of the same organism, so that populations can be normalized by size and age. Furthermore, relationships between accumulations of different heavy metals have been observed (Zn and Hg, As and Pb [1], MeHg and Se [5]), and determination of both MeHg and total heavy metal concentration in individual organisms enables correlations between uptake of different metals to be drawn while excluding intra-species variability. In cases where individual organism size is limited, which is common at lower trophic levels, detection limits are improved when both metal species and total metals are extracted from the whole sample, rather than splitting up the sample for analyses.

Early studies of MeHg in aquatic organisms often relied on extraction methods using bromide salt and copper sulfate saturated acid, followed by isolation in an organic solvent such as toluene, then partitioning into an aqueous complexing reagent such as cysteine or thiosulfate [6,7]. Alkaline digestion in alcohol was later shown to efficiently extract MeHg from tissues, using either KOH in methanol [8,9] or tetramethyl ammonium hydroxide [10]. Recently, a study has shown that a weak acid leach at elevated temperature is also effective in quantitatively extracting MeHg from biological tissue [11]. Derivatized mercury species are then purged onto a solid phase trap, and separated by gas chromatography. Detection has been most frequently reported by atomic fluorescence spectroscopy (AFS) [12], but recently species-specific isotope dilution inductively coupled plasma mass spectrometry (ICP-MS) has also been reported [13]. Total metal quantitation is achieved by oxidizing acid digestion, ideally reaching temperatures of greater than 180°C, followed by determination by ICP-MS. Microwave digestion of samples of aquatic organism tissue has been demonstrated using high-pressure microwave digestion with little loss of volatile elements, such as Se and As [14].

The objective of this work was to expand the acid leach method to allow for determination of Hg species as well as total metals (Zn, As, Se, Cd, Hg, Pb) on low mass samples by ICP-MS. The MeHg extraction was performed first by weak acid leaching and a small volume of extractant was withdrawn for MeHg determination. Additional HNO₃ was then added to further dissolve the sample, and a microwave digestion was performed to allow for total metal quantitation. The original impetus for this method development was to permit total metal determination and Hg speciation for biological samples of limited sample mass (< 10 mg). However, even for samples where sample mass is not limiting there are potential benefits associated with reduced sample preparation time. This method was verified using standard reference materials and was applied to 285 invertebrate and fish samples in a food web study, ranging in sample size from 2 mg to 3 g.

Experimental

Reagents

All sample and standard preparation was performed using ultrapure acids (Optima HNO₃; Fisher Scientific, Pittsburgh, PA, USA) and ultrapure water (>18.2M Ω cm⁻¹) produced by Purelab Pluswater purifier (US Filter, MA, USA). Sodium acetate (3M) was obtained from Fisher Scientific (Pittsburgh, PA, USA), and sodium tetraethylborate from Strem (Newburyport, MA, USA), in 1g bottles. Isotopically enriched mercury isotopes (¹⁹⁹Hg and ²⁰¹Hg) were purchased as Hg(II)O from Oak Ridge National Laboratory, TN, USA. An enriched CH₃-²⁰¹Hg was synthesized in-house as described by Perna et al. [15].

Sample preparation

Four standard reference materials certified for MeHg and the six total metal concentrations (TORT-2 Lobster Hepatopancreas (NRCC, Ottawa, Canada); DORM-2 Dogfish muscle (NRCC, Ottawa, Canada), NIST 1566b oyster tissue (NIST, Gaithersburg, USA), NIST 2976 mussel tissue), as well as a fifth certified only for total metals (Plankton BCR 414 (Geel, Belgium) were used for method validation. Sample weight was varied from 20 to 50 mg for certified reference materials, as well as five replicates of DORM-2 from 5 to 10 mg.

Aquatic invertebrates (39 blue and ribbed mussels, 48 fiddler and green crabs, 29 oysters and clams, 23 sand shrimp, 26 periwinkles, 17 amphipods, 7 polychaete, 19 zooplankton) and small fish (35 killifish, 28 silversides, 5 alewife, 9 stickleback) were collected from six estuaries in New England. Samples were freeze-dried as whole organisms, transferred to 25 mL Teflon screw top vials (machined in-house), with a 15 mm Teflon grinding ball (McMaster Carr, Aurora, OH, USA) added to each sample, and homogenized using a Retsch MM301 ball mill (Haan, Germany). Of the 285 samples included in this study 14 had dry weights of less than 10 mg, and 45 weighed less than 20 mg. Ground samples were stored in glass test tubes (I-Chem, Rockwood, TN, USA) double wrapped in plastic.

A flowchart of the sample preparation protocol is given in Fig. 1. Up to 50 mg of sample was weighed into 7mL PFA Teflon micro-vials (CEM, Matthews, NC, USA). Samples were spiked with an 10 to 50 μL of 50 $\mu\text{g L}^{-1}$ ^{201}Hg and 20 $\mu\text{g L}^{-1}$ ^{199}Hg , depending on sample mass. To each sample was added 2mL 4M HNO_3 , and the weight recorded. For each batch of 32 samples, three digestion blanks with isotopic Hg spikes, and three standard reference materials were prepared. A duplicate sample was also prepared for every 20 samples, where sufficient sample mass was available. Micro-vials were sealed and heated overnight at 55 $^{\circ}\text{C}$. A 50 μL aliquot (100 μL for samples less than 10 mg) of leachate was removed for MeHg determination, then vials were re-weighed, and 1.5 mL concentrated HNO_3 was added to each vessel before re-capping. Two microvessels were placed inside an HP 500 Plus high-pressure microwave vessel containing a spacer and 10 mL of deionized water. Microwave vessels were loaded into a MARS high pressure microwave (CEM), and the temperature was ramped to 150 $^{\circ}\text{C}$ over 20 min., then held at that temperature for another 10 min. Vessels were allowed to cool to room temperature prior to opening. Digested samples were then transferred to 7 mL PTFE tubes (Savillex, Minnetonka, MN, USA) and weighed. Samples were diluted another 5 times for total method determination.

Between batches, vials for milling were cleaned by scrubbing in Citranox (SPI Supplies, West Chester, PA, USA), followed by soaking overnight in 5% HCl :5% HNO_3 , then overnight in 10% HNO_3 , and rinsed with ultrapure water. Microvials for digestion were cleaned by scrubbing with acetone, followed by soaking overnight in 5% HCl :5% HNO_3 . Vials were then rinsed, and filled with 3 mL concentrated HNO_3 and heated to 150 $^{\circ}\text{C}$ in the microwave.

MeHg determination by GC-ICP-MS

Derivatization of MeHg and Hg(II) was performed by aqueous phase ethylation followed by purge and trap, with determination by gas chromatography ICP-MS, in a method similar to that described by Hintelmann and Nguyen [11]. From each digested sample, a 50 μL aliquot was added to 60 mL ultrapure water in a glass reaction and purge vessel (Brooks Rand, Seattle, WA, USA). An aliquot of 0.2 mL 2 M acetate buffer was added to each vessel, resulting in a pH of 4.9. Sodium tetraethylborate solution (50 μL) was added and the vessel was capped for 20 min. Volatile mercury was then collected on a Tenax trap (Tenax TA, mesh 20/35; Alltech, Deerfield, IL, USA) by bubbling with He at a flow-rate of 50 mL min^{-1} for 20 min. The gas flow was then redirected to dry the trap for 5 min., and the trap

was removed and attached to the carrier gas line of the GC-ICP-MS (a similar set-up is depicted in Bloom and Fitzgerald [12]). Mercury was thermally desorbed from the trap by heating to ~ 250 °C for 30 s using a Trap Desorption Module (Brooks Rand). Methyl and inorganic mercury were separated on a Chromosorb W (Sigma-Aldrich, St. Louis, MO, USA) column, prepared in-house by packing a 400×4 mm o.d. U-shaped silanized glass column with quartz wool plugs, held at 85 °C in an adapted drying oven. Mercury species were determined by isotope dilution on an Element 2 ICP-MS (Thermo Finnigan, Bremen, Germany), using operating conditions given in Table 1. Peak areas for MeHg and Hg(II) were integrated and background corrected, and concentrations of MeHg and Hg(II) were derived by species specific isotope dilution using equation 1 given in Point et al.[16].

Determination of total heavy metals by ICP-MS

Heavy metals (Zn, As, Se, Cd, Hg, Pb) were analyzed by collision cell ICP-MS (Agilent 7500c, Santa Clara, CA, USA) using a multi-tune method. The elements Cd, Hg and Pb were analyzed in standard mode (without collision gas), and Zn, As, and Se were measured with He as collision gas, to suppress polyatomic argide and oxide interferences ($^{26}\text{Mg}^{40}\text{Ar}^+$, $^{50}\text{V}^{16}\text{O}^+$, $^{40}\text{Ar}^{35}\text{Cl}^+$, $^{40}\text{Ar}^{37}\text{Cl}^+$). For each run, cycles of four multi-element external calibration standards containing Zn (5 to 500 $\mu\text{g L}^{-1}$), As and Pb (0.5 to 50 $\mu\text{g L}^{-1}$), Se (0.05 to 5 $\mu\text{g L}^{-1}$), Cd (0.01 to 1 $\mu\text{g L}^{-1}$), two blank samples and sixteen samples were analyzed using the operating conditions given in Table 1. All standards and blanks were matrix matched to samples in 1M HNO_3 , and an internal standard containing 30 $\mu\text{g L}^{-1}$ Ge, In, Y and Bi was added in. Analyte count rates were corrected for matrix and drift using the internal standard of the closest mass, and blank-corrected by subtraction of the blank count rates from each cycle. Concentrations were then determined by external calibration then multiplication by the dilution factor, except for Hg, where concentrations were calculated by isotope dilution using the ^{199}Hg and ^{201}Hg spikes.

Results and discussion

When acid digesting biological material for trace element analysis or for Hg speciation it is generally recommended to use a sample mass ≥ 0.25 g. The larger mass minimizes the effects of sample heterogeneity, and improves method detection limits, however, for single aquatic invertebrates or for individual organs of small animals, dry sample weight can be < 10 mg. In such instances microsampling and microdigestion is clearly necessary; furthermore the ability to analyze for both Hg speciation and total metal concentration sequentially on the same sample, rather than splitting the sample, is beneficial particularly when sample mass is limited, since detection limits for both analyses are improved. In our approach we combine the acid leaching method of Hintelmann and Nguyen for extraction of mercury species from tissues using 4M HNO_3 [13] followed by a 9M microwave acid digestion of the remaining extraction suspension to obtain samples for both Hg speciation and total metal analysis.

Method Validation

The method was validated on five standard reference materials. Results for the speciation analysis are presented Table 2a and for total metals in Table 2b. Concentrations of methyl Hg determined by this method were found to be in good agreement with certified values. Recoveries of MeHg for DORM-2, TORT-2, NIST 2976, BCR-14 and NIST 1566b were 96%, 106%, 100%, 93% and 114% with precision, as relative standard deviation, of 5.1%, 2.5%, 0.72%, 0.73% and 2.0% respectively. These reference materials represent a range of MeHg concentrations (0.0132 to 4.24 $\mu\text{g g}^{-1}$) and percent MeHg of total Hg (35 – 91%), suggesting the extraction method is robust for a wide array of samples. The excellent accuracy and precision of this method for methylmercury was also demonstrated in the

original work of Hintelmann and Nguyen for 1566b, TORT-2 and DORM-2 using 20mg samples, and good agreement was also found between MeHg extracted from zooplankton and zoobenthos with 4M HNO₃ and with an alkaline alcohol technique [11]. In the present study, MeHg determined concentration (0.0159) for NIST 1566b (oyster tissue) is slightly higher than range of certified values (0.0125-0.0139), but within a quality assurance range of 20 % of the certified value. Overestimation of MeHg determined in this reference material by a similar analytical method was investigated by Point et al. (2007) and attributed to complexation of methyl groups to the acetic acid buffer [16].

The ethylation and purge and trap methodology followed by GC provides a separation of MeHg from inorganic Hg and thus allows their consecutive quantification in the same chromatographic run. Indeed, one of the original papers by Liang et al. utilizing this methodology with AFS detection reported successful quantification of both Hg species [17]. However, problems associated with decomposition of ethylated species, predominantly during trap heating and desorption, have persisted and are mainly manifested as a loss in recovery of inorganic Hg [18]. Hence, most speciation reports for Hg quantify MeHg only, with inorganic being calculated by subtraction from a separate measurement of total Hg. These indirect 'speciation by difference' methods are inherently less precise than a direct measurement. More importantly for low biomass samples there is often not enough material to perform both a speciation extraction and an acid digestion or, for a composited sample such as zooplankton, the sub-sample variability in total Hg can be quite high thus speciation by difference is not appropriate. A considerable advantage of ICP-MS determination is that species transformation and low recovery can be accounted for by species-specific isotope dilution [16,19,20]. With this technique, the isotope spike reacts in an identical manner to the respective natural Hg species, making the method robust even with decomposition of ethylated species. As can be seen in Table 2a, total Hg results based on the sum of the species for four reference materials are within the 95% confidence limits of the total Hg certified value.

Microwave digestion of the remaining sample solution achieved total metal concentrations in good agreement with certified values (Table 2b). Although digestion temperature was limited to 150 °C by this method due to the temperature and pressure threshold of the micro-digestion vials, sufficient sample decomposition was demonstrated. The average recoveries for Hg, Zn, As, Se, Cd and Pb across 5 CRMs were 97.7%, 99.9%, 103.5%, 100.4%, 103.3 % and 97.1 % respectively, with average precisions (as relative standard deviation) of 7%, 6.7%, 8.7 %, 11.1%, 9.2%, 10.3% respectively. It should be noted that these r.s.d values span a range of analyte concentrations and a range of initial sample masses. The r.s.d. values are somewhat increased due to the small sample masses (2-50 mg) used for the dual extraction and digestion procedure. Nevertheless, the level of accuracy and precision is well within EPA-recommended quality control criteria.

One other study was reported in the literature by Monperrus et al., where measurement of elemental species and total elemental concentrations were achieved in the same initial sample extract [10]. That study also developed a methodology for limited mass samples (benthic macrofauna), but utilized a single extract of the biological tissue with tetramethylammonium hydroxide (TMAOH) with microwave heating for 3 minutes at 40W. The sample was then split for speciation of Hg and Sn by GC-ICP-MS, and for total analysis of V, Cr, Co, Ni, Cu, Zn, As, Ag, Cd and Pb. Method validation was conducted on BCR 278R (mussel tissue) with recoveries of 91%, 109%, 89%, 89% and 92% for Cu, Zn, As, Cd and Pb respectively. Our method provides a good alternative to the Monperrus approach; the use of HNO₃ is more suitable to digestions for 'total' metal analysis because some metals may precipitate in the high pH of the TMAOH procedure and, from a detection limit standpoint, it is relatively easy to obtain HNO₃ in high purity.

Detection Limits—Because Hg species were quantified by isotope dilution, absolute detection limits were calculated from the standard deviation of the ambient MeHg and inorganic Hg concentration determined by isotope dilution in 2 mL spiked extraction blanks [13]. Contributions to Hg background come from reagents, vessels, and traps, and are significantly higher for inorganic Hg than methyl Hg, for which ambient background is close to the instrument background. Ambient MeHg and inorganic Hg were calculated in 15 extraction blanks of 2 mL 4M HNO₃ over several days of analysis. Ambient MeHg was found to be 0.48 $\pm$ 0.47 pg, whereas inorganic Hg, as expected, had a significantly higher ambient blank of 4.3 $\pm$ 3.6 pg. These correspond with method detection limits of 0.072 and 0.54 ng g⁻¹ Hg in 20 mg samples, and propagate to a detection limit of 0.545 ng g⁻¹ for total Hg derived from the sum of Hg species.

Very low detection limits achieved by this method are a product of pre-concentration by purge and trap and the high sensitivity and low instrumental background of open column GC-ICP-MS, and are also affected by the purity of the reagents and low carryover on the vessels and traps. The purge and trap method was developed by Bloom using AFS for detection, achieving very low absolute detection limits (0.6 pg) [8]; the method was demonstrated on alkaline (25% KOH in methanol) digestions of fish tissue, with detection limits of 0.0005 μ g g⁻¹ for MeHg [21]. An advantage to the open column GC technique is that only small (~50 μ L) aliquots of the extracted sample are derivatized, which in this study allowed the remaining sample to be used for total digestion and metal determination. By comparison, capillary GC, which has a significantly smaller column capacity and is therefore restricted to injection volumes of (~1 μ L) [10,15], requires a larger volume of the extracted sample for derivitization to achieve similar detection limits. Montperrus et al. [10] described sequential extraction of MeHg followed by total metal determination, where TMAOH was used as the extractant and Hg species were separated by capillary GC. In that work a larger portion of the extractant (2 out of 5 mL) was ethylated, then extracted and preconcentrated into 50 μ L iso-octane. Very low backgrounds were achieved in that study (0.02 to 0.06 pg for 5 mL TMAH)[10], and similar method detection limits (0.06 ng g⁻¹ for 20 mg sample) to this study were achieved. Using a similar method of acid leaching and open column GC-ICP-MS determination, Hintelmann and Nguyen achieved a slightly higher MeHg background (3 $\pm$ 1 pg MeHg for 5 mL 4M HNO₃) than found in this study [11]. A significant advantage of acid extraction also uncovered in that study was that efficient ethylation could be achieved with larger aliquots of extractant, compared with alkaline digestion where ethylation was impeded at volumes greater than 50 μ L; [11] this is advantageous in the case of very low sample mass, such as in low trophic level species where MeHg concentration is also low, as aliquot size can be augmented as required. For determination of total metal concentrations, instrument detection limits were calculated from 3 times the relative standard deviation of blank samples (containing no analyte) throughout 4 runs (8 blank samples per run). Method detection limits were calculated assuming a sample size of 20 mg, and derived from 3 times the standard deviation of digestion blanks, divided by the slope of the calibration curve. Detection limit for total Hg determined by this method was significantly higher than that for the sum of the species, which is due to marked decrease in sample transport efficiency for solution nebulization (typically < 5%) compared with the high transport efficiency of the GC interface. Should lower detection limits be required for total Hg, cold vapor generation can be used as the sample introduction system, which again provides efficient sample transport efficiency and provides approximately a 10-fold decrease in detection limits compared to solution nebulization. However, for the current purpose, we found that determination of Hg by solution nebulization gave adequate detection limits for the CRMs and samples with the benefit that data are recorded simultaneously for determination of other heavy metals rather than requiring a separate analytical run.

Backgrounds for Zn were significantly higher in digestion blanks than in calibration blanks, but detection limits were still significantly (> 40 times) lower than the concentrations in the samples. For all other elements, there was no significant difference between digestion blanks and calibration blanks. This demonstrates that minimal contamination was caused by the vials or by the microwave digestion procedure, particularly as two micro-digestion vials were heated within each microwave vessel. Determination of Cd, Hg and Pb were performed with no collision gas in the cell, whereas ^{75}As and ^{77}Se were determined with an He flowrate of 3 mL min^{-1} , to remove interference from $^{40}\text{Ar}^{35}\text{Cl}^+$ and $^{40}\text{Ar}^{37}\text{Cl}^+$, respectively. Because no HCl was added to the sample, these argide chloride interferences were found to be minimal, and were sufficiently removed with this low gas flowrate. Detection limits for all metals were sufficient for determination of metals in real samples, with the exception of Cd for which four samples were less than detection limit. Similar instrument detection limits for total Zn, As, Cd and Pb were achieved by TMAOH extraction of aquatic organisms followed by determination by ICP-MS [10], although low recovery was observed for one other metal (Ni). In a study by Enström et al., high resolution ICP-MS was also applied to analysis of fish organ digestions, and achieved detection limits for As in “high” resolution ($m/\Delta m = 10,000$) and Zn in “medium” resolution ($m/\Delta m = 4000$), in the same order of magnitude as were achieved in this study by collision cell ICP-MS [22]. Using low resolution mode, Engström et al. achieved significantly lower (< 10 times) detection limits for Cd and Pb. While speciation analysis in the current work was performed on a double focusing magnetic sector ICP-MS, this instrument is usually reserved for ultratrace analysis, and detection limits for total metals achieved by collision cell ICP-MS were found to be sufficient for this work.

Hg speciation vs. total Hg Validation.—The sum of MeHg and Hg(II) concentrations determined by weak acid leaching followed by GC-ICP-MS, was compared to total Hg concentration determined in the total digestion of the same sample, for 285 aquatic invertebrate and fish samples. A standard least squares analysis of the sum of Hg species vs. total Hg showed good agreement between the two methods of analysis ($y=0.9596x + 0.0019$; Fig. 2). Corroboration between the sum of Hg species concentrations and total Hg concentration on a single sample ensures that Hg is not lost as volatile Hg(0) during total digestion and that complete extraction of Hg is achieved during acid leaching. The latter is particularly important in studies where a variety of organisms are analyzed, as efficient leaching requires homogenization of muscle, skin and exoskeleton. Agreement between the sum of Hg species and the Hg concentration in the digested sample also provides assurance that MeHg is sufficiently extracted from all phases of the sample.

Conclusion

A method for the sequential determination of MeHg and total Zn, As, Se, Cd, Hg, and Pb in a single aquatic organism sample was developed and validated using certified reference materials. Determination of both Hg species and total Hg on the same sample provides quality assurance that complete extraction of Hg species was achieved and that volatile elements were not lost on heating. This procedure is also advantageous in cases where sample mass is low, and where analysis of a single, whole organism is required.

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Fig. 1.
Flowchart of sample preparation procedure for determination of Hg species and total metals.

Fig. 2. Sum of Hg species (mg kg^{-1}) vs. total Hg (mg kg^{-1}), determined sequentially in 285 aquatic organisms. The line represents the least squares regression: $y=0.9596x+0.0019$ $r^2=0.95$; $P<.0001$ Root mean sq error =0.0348.

Table 1

Operating conditions for ICP-MS determination

MeHg determination by ICP-MS (Element 2, Finnigan)	
Rf power	900 W
He carrier gas flow-rate	0.08 L min ⁻¹
Ar make-up gas flow-rate	1.1 L min ⁻¹
Isotopes	¹⁹⁹ Hg, ²⁰⁰ Hg, ²⁰¹ Hg, ²⁰² Hg
Scans	300
Window	60
Dwell	0.02 s
Total metal determination by ICP-MS (7500c, Agilent)	
Rf power	1400 W
Nebuliser gas flow-rate	0.75 L min ⁻¹
Make-up gas flow-rate	0.25 L min ⁻¹
He collision gas	3 mL min ⁻¹
Analyte isotopes (standard mode)	¹¹¹ Cd, ¹¹⁴ Cd, ¹⁹⁹ Hg, ²⁰¹ Hg, ²⁰² Hg, ²⁰⁷ Pb, ²⁰⁸ Pb
Internal standard isotopes	¹¹⁴ In, ²⁰⁹ Bi
Isotopes measure (He mode)	⁶⁶ Zn, ⁶⁸ Zn, ⁷⁵ As, ⁷⁷ Se, ⁸¹ Se
Internal standard isotopes (He mode)	⁷⁴ Ge, ⁸⁹ Y, ¹¹⁴ In
Dwell time	0.02 s
Readings per peak	20
Spray chamber temperature	2 °C

Table 2a

Concentration of MeHg and sum of MeHg and inorganic Hg from speciation analysis, along with certified reference values, for five certified reference materials. All concentrations are in $\mu\text{g g}^{-1}$.

	Speciation extraction GC-ICP-MS			
Sample	MeHg		MeHg + Inorg Hg	
	Measured	Certified	measured	certified
DORM-2 (n=9)	4.24 (± 0.22)	4.47 (± 0.32)	4.64 (± 0.31)	4.64 (± 0.26)
TORT-2 (n=3)	0.161 (± 0.004)	0.152 (± 0.013)	0.297 (± 0.004)	0.27 (± 0.06)
Mussel NIST 2976 (n=5)	0.0279 (± 0.0029)	0.0278 (± 0.0011)	0.0644 (± 0.0083)	0.0610 (± 0.0036)
Plankton BCR-14 (n=3)	0.208 (± 0.008)	0.235 (± 0.007)*	0.272 (± 0.015)	0.276 (± 0.018)
Oyster SRM 1566b (n=5)	0.0159 (± 0.0009)	0.0132 (± 0.0007)	0.0367 (± 0.004)	0.0371 (± 0.0013)

* Value determined by Perna et al. ¹⁵

Table 2b

Determined and certified total Hg, Zn, As, Se, Cd and Pb concentrations in five certified reference materials. All concentrations are in $\mu\text{g g}^{-1}$.

Sample	9M HNO ₃ micro-digestion ICP-MS analysis											
	Total Hg		Zn		As		Se		Cd		Pb	
	measured	certified	measured	certified	measured	certified	measured	certified	measured	certified	measured	certified
DORM-2 (n=8)	4.64 (± 0.31)	4.64 (± 0.26)	24.9 (± 3.9)	25.6 (± 2.3)	18.7 (± 3.0)	18.0 (± 1.1)	1.39 (± 0.33)	1.4 (± 0.09)	0.046 (± 0.007)	0.043 (± 0.008)	0.059 (± 0.013)	0.065 (± 0.007)
TORT-2 (n=4)	0.279 (± 0.026)	0.27 (± 0.06)	174 (± 14)	180 (± 6)	22.8 (± 0.75)	21.6 (± 1.8)	5.37 (± 0.10)	5.63 (± 0.67)	25.8 (± 1.3)	26.7 (± 0.6)	0.315 (± 0.008)	0.35 (± 0.13)
Mussel NIST 2976 (n=5)	0.0566 (± 0.0007)	0.0610 (± 0.0036)	149 (± 8)	137 (± 13)	14.9 (± 2.2)	13.3 (± 1.8)	2.05 (± 0.13)	1.8 (± 0.15)	0.97 (± 0.12)	0.82 (± 0.16)	1.45 (± 0.24)	1.19 (± 0.18)
Plankton BCR-14 (n=3)	0.262 (± 0.013)	0.276($\pm$ 0.018)	106 (± 1)	112 (± 3)	7.10 (± 0.05)	6.82 (± 0.28)	1.62 (± 0.09)	1.75 (± 0.1)	0.359($\pm$ 0.002)	0.383($\pm$ 0.014)	3.41($\pm$ 0.04)	3.97($\pm$ 0.19)
Oyster SRM 1566b (n=5)	0.0361 (± 0.0047)	0.0371 (± 0.0013)	1453 (± 51)	1424 (± 46)	7.05 (± 0.61)	7.65 (± 0.65)	2.08 (± 0.38)	2.06 (± 0.15)	2.50 (± 0.32)	2.48 (± 0.08)	0.299 (± 0.028)	0.308 (± 0.09)

Instrument and method detection limits (based on 20 mg sample size), and median and range of concentrations determined in estuarine invertebrates and fish.

Table 3

	MeHg	Hg(II)	Sum Hg	Total Hg	Zn	As	Se	Cd	Pb
Instrument LOD ($\mu\text{g L}^{-1}$)	1.44 ^a	10.8 ^a	10.9 ^a	0.017	0.36	0.057	0.16	0.005	0.011
Method LOD ($\mu\text{g g}^{-1}$)	0.000072	0.00054	0.00055	0.016	4.5	0.038	0.15	0.009	0.031
Median ($\mu\text{g g}^{-1}$)	0.063	0.028	0.114	0.110	88.89	5.67	2.05	0.342	1.09
Range ($\mu\text{g g}^{-1}$)	0.002-0.302	0.004-1.444	0.011-1.497	0.015-1.123	5.39-16981	0.66-105	0.39-5.7	<0.005-11.53	0.023-36.02

^a Absolute detection limits reported in pg