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Measurement of Mercury Species in Whole Blood Using Speciated Isotope Dilution Methodology Integrated with Microwave-Enhanced Solubilization and Spike Equilibration, Headspace–Solid-Phase Microextraction, and GC-ICP-MS Analysis

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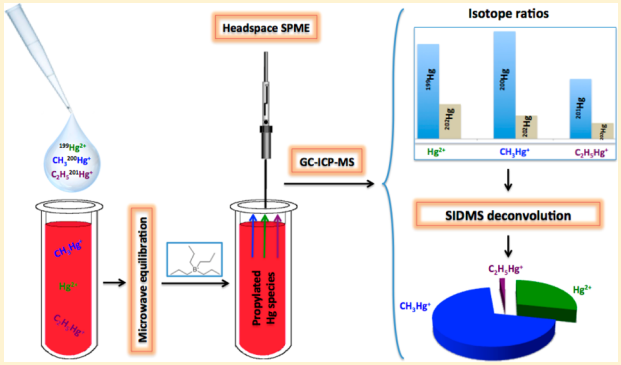
Supporting Information

ABSTRACT: A biomonitoring method was developed for the determination of inorganic-, methyl-, and ethylmercury (Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$, respectively) in whole blood by triple-spiking speciated isotope dilution mass spectrometry (SIDMS) using headspace (HS) solid-phase microextraction (SPME) in combination with gas chromatographic (GC) separation and inductively coupled plasma mass spectrometric (ICP-MS) detection. After spiking the blood sample with isotopically enriched analogues of the analytes ($^{199}Hg^{2+}$, $CH_3{}^{200}Hg^+$ and $C_2H_5{}^{201}Hg^+$), the endogenous Hg species were solubilized in 2.0 mol L^{-1} HNO_3 and equilibrated with the spikes using a microwave-enhanced protocol. The microwaved sample was treated with a 1% (w/v) aqueous solution of sodium tetrapropylborate (buffered to pH 5.2), and the propylated Hg species were sampled in the HS using a Carboxen/polydimethylsiloxane-coated SPME fiber. The extracted species were thermally desorbed from the fiber in the GC injection port and determined by GC-ICP-MS. The analytes were quantified, with simultaneous correction for their method-induced transformation, on the basis of the mathematical relationship in triple-spiking SIDMS. The method was validated using a bovine blood standard reference material (SRM 966, Level 2). Analysis of human blood samples demonstrated the accuracy and reproducibility of the method, which can detect the Hg species down to 30 pg g^{-1} in blood. The validity of the analytical results found for the blood samples was demonstrated using mass balance by comparing the sum of the concentrations of the individual Hg species with the total Hg in the corresponding samples; the latter was determined by isotope dilution mass spectrometry (IDMS) after decomposing the blood using EPA Method 3052 with single-spiking.



Mercury (Hg) has long been known for its adverse health effects that are dependent on its chemical forms. Methylmercury (CH_3Hg^+) readily crosses the blood-brain barrier and causes severe damage on the central nervous system.[1−3] Exposure to inorganic mercury (Hg^{2+}) can result in kidney injury,[1,4] and high levels of elemental mercury (Hg^0) may damage the respiratory, cardiovascular, gastrointestinal, and central nervous systems.[1,3,5] The bioaccumulation of CH_3Hg^+ in fish provides a major route for humans' exposure to the toxicant,[2,3,5] and Hg^{2+} enters the human body through diets high in fructose corn syrup[6] and poultry products in which CH_3Hg^+ (from fish fed to the animals) was metabolized to Hg^{2+}.[3,7] Inorganic Hg is also widely used as an active ingredient in skin-whitening creams in several developing countries.[8] Inhalation of Hg^0 can result from amalgam tooth fillings[2,3,9] and occupational activities.[3,9] Concern also exists regarding exposure to ethylmercury ($C_2H_5Hg^+$) through medical products such as vaccines[2,3] and cleaning solutions for contact lenses[10] that contain thimerosal (sodium ethylmercury thiosalicylate) as a preservative.

Clinical studies mostly cite urine, blood, or hair for assessing humans' exposure to Hg.[3,11] Although urinary Hg excretion is a good indicator of Hg^{2+} and Hg^0,[3,10] it does not provide direct information regarding organomercury species as they are not excreted through urine without changing their chemical forms.[3,5] Both whole blood and hair are suitable to evaluate exposure to CH_3Hg^+,[11] but the potential subject to external contamination (from atmospheric Hg^0, dyes, etc.) argues against hair as a proper medium for monitoring Hg^0 or Hg^{2+}.[3,12] Blood, on the other

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hand, is suitable to assess recent cumulative exposure to Hg[12] because both the elemental,[12] inorganic,[12−14] and organic[12] forms of the element exist in this medium. More than 90% of CH_3Hg^+ concentrates in the red blood cell, and inorganic Hg is accumulated in the erythrocyte fraction and plasma.[12] Elemental Hg has a short life span in blood due to its oxidation to Hg^{2+} by catalase.[15]

Several methods have been reported for the determination of Hg species in samples of various origins.[16,17] Gas chromatography (GC) coupled with inductively coupled plasma mass spectrometry (ICP-MS) has gained a wide use due to the high temporal analyte resolution offered by GC and the high sensitivity, wide dynamic range, and multi-isotopic capabilities of ICP-MS.[16] Derivatizing the analyte species into volatile forms followed by headspace (HS) sampling by solid-phase microextraction (SPME) is an advantageous sample preparation protocol for GC-ICP-MS analysis for several reasons.[18−20] It improves the detection of trace level analytes through preconcentration. The HS sampling enables selective extraction of species that can volatilize at the experimental condition, thereby eliminating the irreversible binding of nonvolatile compounds on the SPME fiber and minimizing the effect of matrix constituents on the separation and detection steps. HS-SPME avoids the use of toxic and expensive organic solvents as the derivatization is carried out in aqueous media. Furthermore, the thermal desorption of analytes in the GC avoids the sample nebulization step in the ICP thereby leading to efficient analyte transport and ionization. HS-SPME-ICP-MS has been used for the determination of Hg^{2+} and/or CH_3Hg^+ in environmental samples[21−23] and in biological[24−27] and environmental reference materials;[27] however, its application has not been reported for the speciation analysis of Hg in human clinical specimens.

Speciation analysis involves a series of processes including sample handling, analyte extraction, separation and detection that can potentially alter the chemical integrity of the target species leading to inaccurate elucidation of their distribution in the original sample. Depending on the sample matrix composition and the analytical processes involved, the Hg species may undergo alkylation, dealkylation, and/or reduction to Hg^0. Such method-induced transformation of Hg species has been reported in human biological specimens,[12,28] fish,[29] soils and sediments,[30] and crude oil.[31] Traditional methods of analyses attempt to preserve the chemical integrity of the target species in several ways that include analyzing the sample immediately after collection and sample preparation, minimizing the sample preparation steps, refrigerating and storing the sample in the dark, and treating the sample with stabilizing reagents. However, these strategies do not guarantee absolute control of the stability of species.

The speciated isotope dilution mass spectrometry (SIDMS) methodology, EPA Method 6800,[32] is uniquely capable to track and correct for in situ transformation of species, thereby enabling accurate and precise determination of analytes. It involves spiking the sample with analogues of the target species tagged with different isotopes of their common central element(s). Sample preparation procedures are applied once the endogenous species are equilibrated with the spiked standards; analyte-spike equilibration is a prerequisite for accuracy in isotope dilution techniques. Samples are analyzed using a mass spectrometer, and analyte concentrations are calculated, with simultaneous correction for in situ transformation of species, by mathematical relationships that use isotopic ratios (not absolute intensity) and known constants without involving calibration curves. The SIDMS procedure also corrects for errors occurring after analyte-spike equilibration[32] that include analyte loss, signal suppression, instrument drift,[32] and imprecision in the HS-SPME sampling that may result from the extremely small size of the extraction phase, inhomogeneity in the polymer surface, degradation of the fiber, displacement of analyte(s), and variation in derivatization. The fundamentals of SIDMS are discussed in patents,[33,34] EPA Method 6800,[32] and a book chapter,[35] and its applications have been demonstrated in several publications from the authors' research group.[28−31]

The goal of the present study was to develop a method for the comprehensive speciation analysis of Hg in whole blood using HS-SPME-GC-ICP-MS with triple-spiking speciated isotope dilution methodology. To the best of the authors' knowledge, such a strategy has not been reported for the analysis of human biological specimens. In the proposed method, the blood samples were spiked with known amounts of isotopically enriched analogues of the target species (i.e., $^{199}Hg^{2+}$, $CH_3{}^{200}Hg^+$, and $C_2H_5{}^{201}Hg^+$), and the endogenous species were solubilized and equilibrated with the spiked species using a microwave-enhanced protocol. The analyte and spiked species in the microwaved sample were derivatized to volatile forms, HS sampled using SPME, and determined by GC-ICP-MS. The endogenous Hg species in the blood were quantified using the mathematical relationships in triple-spiking SIDMS based on the isotopic ratios of the analytes derived from their chromatographic peak areas. A bovine blood standard reference material (SRM 966, Level 2) was used to validate the protocol. Human blood samples drawn from five subjects were analyzed, and the validity of the analytical results was evaluated using mass balance by comparing the sum of the measured concentrations of the individual Hg species with the total concentration of Hg found in the corresponding samples; the latter was determined by isotope dilution mass spectrometry (IDMS) after spiking the blood samples with ^{199}Hg and decomposing them using mineral acid in a microwave oven.

EXPERIMENTAL SECTION

Instrumentation and Software. Extraction and sample decomposition were performed using a closed vessel laboratory microwave oven (Ethos 1, Milestone). The instrument was equipped with temperature and pressure feedback control and sample stirring. The device senses within ±2.0 °C of the target temperature and automatically adjusts the microwave field output power. Teflon microwave vessels were used for sample decomposition, and extractions were carried out using quartz vessels.

A model 7890A gas chromatograph and a model 7700 ICP-MS (both from Agilent Technologies) were used. The GC capillary column was Agilent J&W DB-5 (30 m long and 0.25 mm i.d.) coated with a 0.25 μm thick film of cross-linked and surface bonded (5%-phenyl)-methyl-polysiloxane. The gas chromatograph was equipped with an interface for coupling with ICP-MS; the interface had a demountable torch with heated injector and transfer line. Ultra high purity grade helium (99.999%, Airgas) was used as a carrier gas at a flow rate of 2 mL min^{-1}. The injection port and the transfer line were kept at 150 °C. The column temperature program was (a) 60 °C, hold for 3 min, (b) ramp to 120 °C at 15 °C min^{-1}, and (c) ramp to 220 °C at 25 °C min^{-1}, hold for 1 min. The operating conditions of the ICP-MS were RF power (1550 W), RF matching (1.8 V), sampling depth (8 and 10 mm for ICP-MS and GC-ICP-MS, respectively),

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plasma gas flow (15 L min^{-1}), carrier gas flow (0.95 L min^{-1}), dilution gas flow (0.15 and 0.25 L min^{-1} for ICP-MS and GC-ICP-MS, respectively), and spray chamber temperature (2 °C for ICP-MS). Ultra high purity grade (99.999%, Airgas) argon was used. For direct analysis by ICP-MS, the instrument was tuned using an Agilent tuning solution containing 1 $\mu g\ L^{-1}$ Li, Co, Y, Ce, and Tl in 2% HNO_3, and samples were introduced using an autosampler (ASX-500, Agilent Technologies) kept in an anticontamination enclosure (ENC500, CETAC Technologies). For the GC-ICP-MS configuration, instrument tuning was made using 0.1% Xe in He.

A Stableflex/SS SPME fiber core coated with 85 μm thick Carboxen/polydimethylsiloxane (Car/PDMS) film (Supelco) was used for HS sampling of the derivatized (propylated) Hg species. The SPME fiber was manually inserted into the GC, and injection was made in splitless mode.

Agilent MassHunter software (version G7201A A.01.01, Agilent Technologies) was used for ICP-MS data acquisition and chromatographic peak integration. Raw data were exported into a Microsoft Excel compatible format to calculate isotope ratios. Hg-SPC software in the Hg speciation analysis kit from Applied Isotope Technologies was used for analyte quantification by IDMS and SIDMS.

Chemicals and Standards. Ultra trace grade HNO_3 (69%, Fisher Scientific), 30% H_2O_2 (EMD), HPLC grade glacial acetic acid (Fisher Scientific), ammonium acetate (Fisher Scientific), sodium (tetra-n-propyl) borate ($NaB(C_3H_7)_4$, 99%, ABCR Gmbh & CO), disodium salt of ethylenediaminetetracetic acid (EDTA, Fisher Scientific), and ultrapure water (18.2 MΩ cm, Branstead NANOpure) were used. All the reagents were of analytical grade.

Stock solutions of natural abundant (Nat) and isotopically enriched Hg species (i.e., $^{Nat}HgCl_2$, $CH_3{}^{Nat}HgCl$, $C_2H_5{}^{Nat}HgCl$, $^{199}HgCl_2$, $CH_3{}^{200}HgCl$ and $C_2H_5{}^{201}HgCl$) were provided in the Hg speciation analysis kit by Applied Isotope Technologies. The $^{199}HgCl_2$, $CH_3{}^{200}HgCl$, and $C_2H_5{}^{201}HgCl$ standards were enriched with 91.95% ^{199}Hg, 96.41% ^{200}Hg, and 98.11% ^{201}Hg, respectively. Working solutions were prepared by diluting appropriate amounts of the stock solutions in water; all measurements were made by mass with 0.00001 g precision. All the Hg standard solutions were stored in amber glass bottles at 4 °C, away from ultraviolet lamp and sunlight.

Blood Samples and Standard Reference Material. Blood samples (HB1−HB5) were collected from five healthy individuals with no known record of occupational exposure to Hg. Samples HB1, HB2, and HB3 were collected from subjects who had no dietary restrictions, whereas HB4 and HB5 were collected from nonfish eaters. Sample HB1 was drawn 24 h after administering a flu vaccine to the subject. Blood was drawn from the donor's vein into three trace-metal-free drawtubes containing anticoagulant (EDTA) and immediately stored at −20 °C. SRM 966 (Toxic metals in bovine blood, Level 2) from National Institute of Standards and Technology (NIST) was used for method validation. All procedures pertaining to sample preparation, storage, and analysis were carried out in a clean room equipped with a class-100 high efficiency particulate air filter hood.

Sample Decomposition and Analysis for Total Hg Quantitation. The blood samples were decomposed based on EPA Method 3052[36] integrated with the IDMS component of EPA Method 6800.[32] Each sample was thawed to room temperature, and 2 g portions were weighed out into three Teflon microwave vessels followed by spiking with appropriate amount of $^{199}Hg^{2+}$ standard solution (quantified by mass); the optimum analyte-to-spike ratio for Hg ($^{202}Hg/^{199}Hg$, $^{202}Hg/^{200}Hg$, and $^{202}Hg/^{201}Hg$) spreads in the range 0.01−1.0.[28] Concentrated HNO_3 (5.0 mL) and 1.0 mL of 30% H_2O_2 were added into each vessel, and the mixtures were swirled to ensure mixing. After putting Teflon-coated magnetic bars, the vessels were sealed and irradiated in the microwave system at 180 °C for 10 min with a ramp time of 5 min.[36] The digests were cooled to room temperature, transferred into clean amber glass bottles, diluted to 10 mL with ultrapure water, and stored in a clean cold room at 4 °C until analysis (same or next day). Three procedural blanks were prepared.

The digests were analyzed by ICP-MS in spectrum mode after a 100-fold dilution with 1% HNO_3. Each digest was analyzed four times.

Microwave-Enhanced Solubilization of Analytes. Analyte species were solubilized from the blood in a microwave-enhanced protocol based on EPA Method 3200[37] integrated with triple-spiking SIDMS.[32] Each blood sample was thawed to room temperature, and 0.5 g portions were weighed out into three quartz microwave vessels with Teflon-coated magnetic bars inside. The samples were spiked with appropriate amounts of $^{199}Hg^{2+}$, $CH_3{}^{200}Hg^+$, and $C_2H_5{}^{201}Hg^+$ standards (quantified by mass), followed by addition of 7.2 mL of 2.0 mol L^{-1} HNO_3. Each vessel was swirled to ensure mixing, capped, and carefully placed in a large Teflon microwave vessel in which 10 mL of 2.0 mol L^{-1} HNO_3 had been added. The large vessels were sealed and irradiated in the microwave oven at 100 °C for 10 min with a ramp time of 2 min. The samples were cooled to ambient temperature and centrifuged at 4000 rpm for 20 min. The supernatants were carefully decanted into clean amber glass bottles and stored in a clean cold room at 4 °C until analysis (same or next day). Three procedural blanks were prepared.

Analyte Derivatization, HS-SPME Sampling, and GC-ICP-MS Analysis. The endogenous and spiked Hg species in the microwaved samples were derivatized to volatile forms through propylation as follows. Each sample was taken out from the cold room and kept in ice. A 2.0 mL portion of the cold sample was transferred into a 20 mL glass vial (having a polytetrafluoroethylene-coated silicon rubber septum) with a Teflon-coated magnetic stir bar inside. Eight milliliters of acetate buffer (0.287 mol L^{-1} acetic acid and 0.710 mol L^{-1} ammonium acetate, pH 5.2) and 0.1 mL of 1% (w/v) aqueous $NaB(C_3H_7)_4$ solution were immediately added into the vial. The propylation solution was prepared fresh on the day of every analysis and kept in ice throughout. The vial was capped, and the mixture was stirred for 3−5 min at room temperature. The SPME needle was inserted into the HS through the septum, and the vial was placed in a water bath (65 °C). The solution was stirred continuously while the volatile derivatives of the target species were sampled in the HS for 10 min. The SPME fiber was removed from the vial and immediately inserted into the GC injection port, which was heated at 150 ° C. The analytes were desorbed from the fiber in 60 s, and the GC-ICP-MS analysis was conducted in time-resolved analysis (TRA) mode. The HS-SPME-GC-ICP-MS procedure was repeated four times for each microwaved sample.

Mass Bias Correction. The analytical data were corrected for mass bias. Mass bias correction factors were determined by analyzing a 25.0 ng g^{-1} $^{Nat}Hg^{2+}$ solution prepared in the digestion blank (ICP-MS) and a solution containing $^{Nat}Hg^{2+}$, $CH_3{}^{Nat}Hg^+$, $C_2H_5{}^{Nat}Hg^+$ (10.0 ng g^{-1} Hg per specie) prepared in 2.0 mol L^{-1}

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HNO_3 (GC-ICP-MS). The solutions were analyzed in triplicate at the beginning, middle, and end of each analysis sequence.

Safety Considerations. Methylmercury and derivatized organomercury species are highly toxic and must be handled with appropriate personal protection. Material safety data sheets were consulted, and essential safety precautions were employed for manipulations of all chemicals and reagents.

RESULTS AND DISCUSSION

Solubilization of the Hg Species from the Blood Samples. Several protocols have been reported for solubilizing blood Hg that use either acidic[10,38−42] or alkaline[10,43−45] solutions containing $CuSO_4$ and/or KBr,[10,39,42] diethyldithiocarbamate,[38] L-cysteine and 2-mercaptoethanol,[40,41] KOH,[10,44,45] tetramethylammonium hydroxide,[43] or other reagents with[10,38,39,42,44,45] or without[40,41,43] subsequent extraction into organic solvents. In the present study, a 2.0 mol L^{-1} HNO_3 solution was used with microwave-enhanced heating (see experimental section) as it provides several advantages. HNO_3 cleaves the attachment between Hg and thiol groups without affecting the Hg−C bond. The solubilization procedure is simple and fast, and it avoids extensive use of reagents. Furthermore, the use of a microwave enables to achieve instantaneous equilibration between the spiked and the endogenous analytes,[46,47] and the risk of losing volatile Hg compounds was avoided by using a closed microwave system. The final aqueous microwaved solution is suitable for derivatizing the analyte species for subsequent sampling by SPME in the HS.

Derivatization of Hg Species to Volatile Forms. Tetraethyl-, tertapropyl-, and tetraphenyl-borates are used to derivatize the species of Hg for HS-SPME sampling, as the reagents are suitable for use in aqueous media.[48] The use of tetraethylborate, however, does not allow the simultaneous determination of Hg^{2+} and $C_2H_5Hg^+$ as the two species become indistinguishable after ethylation. Additionally, the derivatization solution lacks stability,[49] and the reagent transforms CH_3Hg^+ into Hg^0 in halide-containing media.[50] Phenylation suffers from long equilibration time and low extraction efficiency.[49]

In the present study, the analyte species (Hg^{2+}, CH_3Hg^+ and $C_2H_5Hg^+$) were propylated using $NaB(C_3H_7)_4$ to form $Hg(C_3H_7)_2$, $CH_3HgC_3H_7$ and $C_2H_5HgC_3H_7$, respectively. Propylation allows the determination of $C_2H_5Hg^+$ along with other Hg species. The derivatization reaction is efficient and fast yielding stable products.[10,43,51] The pH of the derivatization medium was adjusted to 5.2 as reported elsewhere.[51] An amount of $NaB(C_3H_7)_4$ solution (see experimental section) which gives 0.01% (w/v) $NaB(C_3H_7)_4$ in the derivatization mixture was used to minimize the interference of $B(C_3H_7)_3$ on the transfer of the propylated Hg species to the HS and on their subsequent sorption by the SPME fiber. Such interference has been reported previously.[27]

Sampling of the Volatile Derivatives in the HS Using SPME. HS-SPME is affected by a number of factors including the sorbent type, analyte equilibration between the HS and the SPME fiber, and temperature and duration of the HS sampling. Because studies showed that mixed-coating fibers yield much better extraction efficiency than PDMS for shorter alkyl-chain organometallic compounds,[27] a Car/PDMS fiber was used in the present study. The derivatization mixture was kept at 65 °C, as moderately elevated temperatures increase the partial vapor pressure of the analytes in the HS.[48] To achieve fast analyte equilibration, the derivatization mixture was stirred continuously while the volatiles were sampled from the HS. The mixture was also stirred for 3−5 min prior to introducing the fiber.

Effect of Injection Temperature on Analyte Desorption. The GC injector temperature is a critical factor because high temperatures may decompose the analytes and the SPME fiber coating, and low temperatures can lead to inefficient analyte desorption, which results in sample carryover and peak tailing. In the present study, the effect of the injection port temperature on the analytes' desorption was studied in the temperature range of 120 to 250 °C. Formation of Hg^0 was observed for temperatures higher than 180 °C. Figure 1 shows a chromatogram for the GC-ICP-MS analysis of a standard solution at an injector temperature of 200 °C. All the propylated Hg species (i.e., $Hg(C_3H_7)_2$, $CH_3HgC_3H_7$, and $C_2H_5HgC_3H_7$) were degraded to form Hg^0; the highest degradation was for propylated Hg^{2+} ($Hg(C_3H_7)_2$), as indicated by the dominant $^{199}Hg^0$ and weak $^{199}Hg^{2+}$ peaks in the chromatogram (Figure 1). Previous studies also reported the thermal degradation of alkylated Hg species to Hg^0 at injector temperatures higher than 150 °C[52] and 170 °C.[53] In the present study, an injection port temperature of 150 °C for 60 s assured desorption of all the analytes without causing degradation and carryover on any of the species.

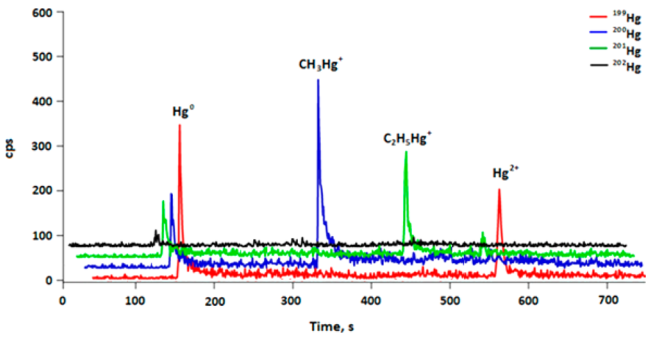

Figure 1. GC-ICP-MS chromatogram for a standard solution containing 25 ng g^{-1} (as Hg) of Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$ after HS sampling of the propylated species using SPME. The GC injection port temperature was 200 °C; see the experimental section for the other GC-ICP-MS conditions. Baselines are shifted for clarity.

The column temperature was optimized thoroughly, and the program described in the experimental section provided baseline separation of the species with sharp and symmetrical peaks in less than 10 min. Figure 2 shows a chromatogram for a blood sample spiked with the isotopically enriched Hg species.

Analyte Quantification. Two mathematical approaches were used for analyte quantification. The concentration of total Hg in the blood samples was determined by IDMS using the data obtained from the analysis of the corresponding digests by ICP-MS. The IDMS equations are described elsewhere.[32] Triple-spiking SIDMS equations were used to calculate the concentrations of Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$, with simultaneous correction for their in situ transformation(s) using the data obtained from the analysis of the blood samples by GC-ICP-MS after microwave-enhanced preparation and HS-SPME. The SIDMS equations for triple-spiking SIDMS are described in the following section; the equations have not been discussed in the literature previously. In both calculations (IDMS and SIDMS), the isotopic abundance of ^{202}Hg was used to monitor the endogenous Hg species.

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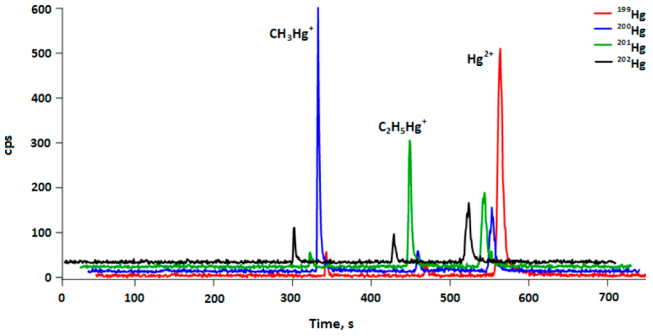

Figure 2. GC-ICP-MS chromatogram for a blood sample spiked with $^{199}Hg^{2+}$, $CH_3{}^{200}Hg^+$, and $C_2H_5{}^{201}Hg^+$. The blood was microwaved and HS-SPME sampled according to the conditions described in the experimental section. Baselines are shifted for clarity.

To achieve high accuracy and precision, all quantifications (IDMS and SIDMS) were made using mass bias corrected isotope ratios as described in EPA Method 6800.[32] The effect of interferences on the Hg isotopes was evaluated by comparing the mass bias corrected isotopic ratios with the expected values. Ratios of 0.5679, 0.7742, and 0.4437 were obtained for $^{199}Hg/^{202}Hg$, $^{200}Hg/^{202}Hg$, and $^{201}Hg/^{202}Hg$, respectively, in a standard solution containing 25 ng g^{-1} (as Hg) of each of the three Hg species in 2% HNO_3. The ratios were in very good agreement with the IUPAC values (i.e., 0.5649, 0.7736, and 0.4414, respectively).[54]

Algorithms, Assumptions, and Calculations in Triple-Spiking SIDMS. Consider an aqueous sample containing Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$ with concentrations (μmol g^{-1}) of $C_x^{Hg^{2+}}$, $C_x^{CH_3Hg^+}$, and $C_x^{C_2H_5Hg^+}$, respectively. Weigh out W_x gram of the sample, and spike it with $W_s^{Hg^{2+}}$, $W_s^{CH_3Hg^+}$, and $W_s^{C_2H_5Hg^+}$ grams of $^{199}Hg^{2+}$, $CH_3{}^{200}Hg^+$, and $C_2H_5{}^{201}Hg^+$ standards of concentrations (μmol/g) $C_s^{Hg^{2+}}$, $C_x^{CH_3Hg^+}$, and $C_s^{C_2H_5Hg^+}$, respectively. If there is no transformation of species in the sample, the concentrations of the analytes after spiking will be the following: (1) $^{199}A_xC_x^{Hg^{2+}}W_x + {}^{199}A_s^{Hg^{2+}}C_x^{Hg^{2+}}W_s^{Hg^{2+}}$ μmol of ^{199}Hg as Hg^{2+}, (2) $^{199}A_xC_x^{CH_3Hg^+}W_x + {}^{199}A_s^{CH_3Hg^+}C_s^{CH_3Hg^+}W_s^{CH_3Hg^+}$ μmol of ^{199}Hg as CH_3Hg^+, and (3) $^{199}A_xC_x^{C_2H_5Hg^+}W_x + {}^{199}A_s^{C_2H_5Hg^+}C_s^{C_2H_5Hg^+}W_s^{C_2H_5Hg^+}$ μmol of ^{199}Hg as $C_2H_5Hg^+$, where "A" represents the abundance of the Hg isotope under consideration.

If the species undergo bidirectional transformations (as described in Figure 3) after the spiked species equilibrate with

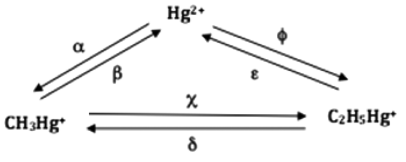

Figure 3. Interconversion pathways among the three Hg species: Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$.

the endogenous analytes, the above number of moles of the species will change.

Let α, β, χ, δ, ε, and ϕ represent the fractions for the conversion of Hg^{2+} to CH_3Hg^+, CH_3Hg^+ to Hg^{2+}, CH_3Hg^+ to $C_2H_5Hg^+$, $C_2H_5Hg^+$ to CH_3Hg^+, $C_2H_5Hg^+$ to Hg^{2+}, and Hg^{2+} to $C_2H_5Hg^+$, respectively.

After interconversions among Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$, the total amount of ^{199}Hg in Hg^{2+} form becomes

$$({}^{199}A_xC_x^{Hg^{2+}}W_x + {}^{199}A_s^{Hg^{2+}}C_x^{Hg^{2+}}W_s^{Hg^{2+}})(1-\alpha-\phi) + ({}^{199}A_xC_x^{CH_3Hg^+}W_x + {}^{199}A_s^{CH_3Hg^+}C_s^{CH_3Hg^+}W_s^{CH_3Hg^+})\beta + ({}^{199}A_xC_x^{C_2H_5Hg^+}W_x + {}^{199}A_s^{C_2H_5Hg^+}C_s^{C_2H_5Hg^+}W_s^{C_2H_5Hg^+})\varepsilon$$

Similarly, the total amount of ^{199}Hg in CH_3Hg^+ form becomes

$$({}^{199}A_xC_x^{Hg^{2+}}W_x + {}^{199}A_s^{Hg^{2+}}C_s^{Hg^{2+}}W_s^{Hg^{2+}})\alpha + ({}^{199}A_xC_x^{CH_3Hg^+}W_x + {}^{199}A_s^{CH_3Hg^+}C_s^{CH_3Hg^+}W_s^{CH_3Hg^+})(1-\beta-\chi) + ({}^{199}A_xC_x^{C_2H_5Hg^+}W_x + {}^{199}A_s^{C_2H_5Hg^+}C_s^{C_2H_5Hg^+}W_s^{C_2H_5Hg^+})\delta$$

and the total amount of ^{199}Hg in $C_2H_5Hg^+$ form becomes

$$({}^{199}A_xC_x^{Hg^{2+}}W_x + {}^{199}A_s^{Hg^{2+}}C_s^{Hg^{2+}}W_s^{Hg^{2+}})\varphi + ({}^{199}A_xC_x^{CH_3Hg^+}W_x + {}^{199}A_s^{CH_3Hg^+}C_s^{CH_3Hg^+}W_s^{CH_3Hg^+})\chi + ({}^{199}A_xC_x^{C_2H_5Hg^+}W_x + {}^{199}A_s^{C_2H_5Hg^+}C_s^{C_2H_5Hg^+}W_s^{C_2H_5Hg^+})(1-\delta-\varepsilon)$$

Similar equations can be constructed for ^{200}Hg, ^{201}Hg and ^{202}Hg resulting in a total of 12 equations for the four isotopes of Hg. The 12 equations can be transformed into the nine isotope ratio (R) equations given in Chart 1 (next page) by dividing the ^{199}Hg, ^{200}Hg and ^{201}Hg equations by the corresponding ^{202}Hg equations. In the equations (Chart 1), $R_{199/202}^{Hg^{2+}}$, $R_{200/202}^{Hg^{2+}}$, $R_{201/202}^{Hg^{2+}}$, $R_{199/202}^{CH_3Hg^+}$, $R_{200/202}^{CH_3Hg^+}$, $R_{201/202}^{CH_3Hg^+}$, $R_{199/202}^{C_2H_5Hg^+}$, $R_{200/202}^{C_2H_5Hg^+}$, and $R_{201/202}^{C_2H_5Hg^+}$, represent the ratios of Hg isotopes for $^{199}Hg^{2+}/^{202}Hg^{2+}$, $^{200}Hg^{2+}/^{202}Hg^{2+}$, $^{201}Hg^{2+}/^{202}Hg^{2+}$, $CH_3{}^{199}Hg^+/CH_3{}^{202}Hg^+$, $CH_3{}^{200}Hg^+/CH_3{}^{202}Hg^+$, $CH_3{}^{201}Hg^+/CH_3{}^{202}Hg^+$, $C_2H_5{}^{199}Hg^+/C_2H_5{}^{202}Hg^+$, $C_2H_5{}^{200}Hg^+/C_2H_5{}^{202}Hg^+$, and $C_2H_5{}^{201}Hg^+/C_2H_5{}^{202}Hg^+$ in the spiked sample, respectively; $^{199}A_x$, $^{200}A_x$, $^{201}A_x$, and $^{202}A_x$ are the natural relative isotopic abundances of ^{199}Hg, ^{200}Hg, ^{201}Hg, and ^{202}Hg in the original (unspiked) sample, respectively; $^{199}A_s^{Hg^{2+}}$, $^{200}A_s^{Hg^{2+}}$, $^{201}A_s^{Hg^{2+}}$, $^{202}A_s^{Hg^{2+}}$, $^{199}A_s^{CH_3Hg^+}$, $^{200}A_s^{CH_3Hg^+}$, $^{201}A_s^{CH_3Hg^+}$, $^{202}A_s^{CH_3Hg^+}$, $^{199}A_s^{C_2H_5Hg^+}$, $^{200}A_s^{C_2H_5Hg^+}$, $^{201}A_s^{C_2H_5Hg^+}$, and $^{202}A_s^{C_2H_5Hg^+}$ are the relative isotopic abundances of ^{199}Hg (in $^{199}Hg^{2+}$), ^{200}Hg (in $^{199}Hg^{2+}$), ^{201}Hg (in $^{199}Hg^{2+}$), ^{202}Hg (in $^{199}Hg^{2+}$), ^{199}Hg (in $CH_3{}^{200}Hg^+$), ^{200}Hg (in $CH_3{}^{200}Hg^+$), ^{201}Hg (in $CH_3{}^{200}Hg^+$), ^{202}Hg (in $CH_3{}^{200}Hg^+$), ^{199}Hg (in $C_2H_5{}^{201}Hg^+$), ^{200}Hg (in $C_2H_5{}^{201}Hg^+$), ^{201}Hg (in $C_2H_5{}^{201}Hg^+$), and ^{202}Hg (in $C_2H_5{}^{201}Hg^+$), respectively; $C_x^{Hg^{2+}}$, $C_x^{CH_3Hg^+}$, and $C_x^{C_2H_5Hg^+}$ are the concentrations (μmol g^{-1}) of Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$ in the original (unspiked) sample.

The nine isotope ratio (R) equations have 18 unknowns, i.e., isotopic ratios of $^{199}Hg/^{202}Hg$, $^{200}Hg/^{202}Hg$, and $^{201}Hg/^{202}Hg$ for the three Hg species, $C_x^{Hg^{2+}}$, $C_x^{CH_3Hg^+}$, $C_x^{C_2H_5Hg^+}$, α, β, ϕ, ε, χ, and δ. Because the isotopic ratios for the three species can be measured experimentally, nine unknowns will remain in the nine equations that can be solved for the original concentrations of Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$ and the fractions of interconversions among the three species (i.e., α, β, ϕ, ε, χ, and δ).

Analysis of Blood Samples. Blood samples collected from five individuals (Table 1) were analyzed for their total and individual Hg species. SRM 966, Level 2 was used for method validation.

Determination of Total Hg. The total concentration of Hg in the samples was determined by IDMS as described in the experimental section. The digests of the spiked blood samples (with ^{199}Hg) were analyzed by ICP-MS; ion counts (cps) were recorded at m/z 199 and 202. IDMS calculations were made

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Chart 1. Isotope ratio equations for Hg^+, CH_3Hg^+, and $C_2H_5Hg^+$.

Isotope ratio equations for Hg^{2+}

$$R^{Hg^{2+}}_{199/202} = \frac{\left({}^{199}A_x C_x^{Hg^{2+}} W_x + {}^{199}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)(1-\alpha-\phi) + \left({}^{199}A_x C_x^{CH_3Hg^+} W_x + {}^{199}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\beta + \left({}^{199}A_x C_x^{C_2H_5Hg^+} W_x + {}^{199}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\varepsilon}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)(1-\alpha-\phi) + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\beta + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\varepsilon}$$

$$R^{Hg^{2+}}_{200/202} = \frac{\left({}^{200}A_x C_x^{Hg^{2+}} W_x + {}^{200}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)(1-\alpha-\phi) + \left({}^{200}A_x C_x^{CH_3Hg^+} W_x + {}^{200}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\beta + \left({}^{200}A_x C_x^{C_2H_5Hg^+} W_x + {}^{200}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\varepsilon}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)(1-\alpha-\phi) + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\beta + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\varepsilon}$$

$$R^{Hg^{2+}}_{201/202} = \frac{\left({}^{201}A_x C_x^{Hg^{2+}} W_x + {}^{201}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)(1-\alpha-\phi) + \left({}^{201}A_x C_x^{CH_3Hg^+} W_x + {}^{201}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\beta + \left({}^{201}A_x C_x^{C_2H_5Hg^+} W_x + {}^{201}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\varepsilon}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)(1-\alpha-\phi) + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\beta + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\varepsilon}$$

Isotope ratio equations for CH_3Hg^+

$$R^{CH_3Hg^+}_{199/202} = \frac{\left({}^{199}A_x C_x^{Hg^{2+}} W_x + {}^{199}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\alpha + \left({}^{199}A_x C_x^{CH_3Hg^+} W_x + {}^{199}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)(1-\beta-\chi) + \left({}^{199}A_x C_x^{C_2H_5Hg^+} W_x + {}^{199}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\delta}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\alpha + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)(1-\beta-\chi) + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\delta}$$

$$R^{CH_3Hg^+}_{200/202} = \frac{\left({}^{200}A_x C_x^{Hg^{2+}} W_x + {}^{200}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\alpha + \left({}^{200}A_x C_x^{CH_3Hg^+} W_x + {}^{200}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)(1-\beta-\chi) + \left({}^{200}A_x C_x^{C_2H_5Hg^+} W_x + {}^{200}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\delta}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\alpha + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)(1-\beta-\chi) + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\delta}$$

$$R^{CH_3Hg^+}_{201/202} = \frac{\left({}^{201}A_x C_x^{Hg^{2+}} W_x + {}^{201}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\alpha + \left({}^{201}A_x C_x^{CH_3Hg^+} W_x + {}^{201}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)(1-\beta-\chi) + \left({}^{201}A_x C_x^{C_2H_5Hg^+} W_x + {}^{201}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\delta}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\alpha + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)(1-\beta-\chi) + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)\delta}$$

Isotope ratio equations for $C_2H_5Hg^+$

$$R^{C_2H_5Hg^+}_{199/202} = \frac{\left({}^{199}A_x C_x^{Hg^{2+}} W_x + {}^{199}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\phi + \left({}^{199}A_x C_x^{CH_3Hg^+} W_x + {}^{199}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\chi + \left({}^{199}A_x C_x^{C_2H_5Hg^+} W_x + {}^{199}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)(1-\delta-\varepsilon)}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\phi + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\chi + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)(1-\delta-\varepsilon)}$$

$$R^{C_2H_5Hg^+}_{200/202} = \frac{\left({}^{200}A_x C_x^{Hg^{2+}} W_x + {}^{200}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\phi + \left({}^{200}A_x C_x^{CH_3Hg^+} W_x + {}^{200}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\chi + \left({}^{200}A_x C_x^{C_2H_5Hg^+} W_x + {}^{200}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)(1-\delta-\varepsilon)}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\phi + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\chi + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)(1-\delta-\varepsilon)}$$

$$R^{C_2H_5Hg^+}_{201/202} = \frac{\left({}^{201}A_x C_x^{Hg^{2+}} W_x + {}^{201}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\phi + \left({}^{201}A_x C_x^{CH_3Hg^+} W_x + {}^{201}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\chi + \left({}^{201}A_x C_x^{C_2H_5Hg^+} W_x + {}^{201}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)(1-\delta-\varepsilon)}{\left({}^{202}A_x C_x^{Hg^{2+}} W_x + {}^{202}A_s^{Hg^{2+}} C_s^{Hg^{2+}} W_s^{Hg^{2+}}\right)\phi + \left({}^{202}A_x C_x^{CH_3Hg^+} W_x + {}^{202}A_s^{CH_3Hg^+} C_s^{CH_3Hg^+} W_s^{CH_3Hg^+}\right)\chi + \left({}^{202}A_x C_x^{C_2H_5Hg^+} W_x + {}^{202}A_s^{C_2H_5Hg^+} C_s^{C_2H_5Hg^+} W_s^{C_2H_5Hg^+}\right)(1-\delta-\varepsilon)}$$

Table 1. Concentrations (ng g^{-1}) of Total Hg and Individual Hg Species Determined in SRM 996 and Human Blood Samples by IDMS and SIDMS, Respectively, and Recovery (%) Values (n = 12, 95% CL)

sample	Hg (total)[a]	Hg species[b]			sum of Hg species	recovery (%)[c]
		Hg^{2+}	CH_3Hg^+	$C_2H_5Hg^+$		
SRM 966 Level 2, bovine blood	31.9 ± 2.1 (31.4 ± 1.7)	16.7 ± 1.2 (14.87 ± 0.93)	15.3 ± 1.5 (16.4 ± 1.4)	ND	32.0 ± 2.7	100.3
HB1	5.2 ± 0.56	1.5 ± 0.12	3.7 ± 0.48	0.09 ± 0.02	5.3 ± 0.62	101.9
HB2	30.6 ± 3.2	5.4 ± 1.5	25.2 ± 2.8	ND	30.6 ± 4.3	100.0
HB3	23.4 ± 2.3	4.3 ± 2.3	19.1 ± 2.3	ND	23.4 ± 4.6	100.0
HB4	5.6 ± 1.85	5.9 ± 1.3	ND	ND	5.9 ± 1.3	105.4
HB5	0.47 ± 0.05	0.52 ± 0.14	ND	ND	0.52 ± 0.14	110.6

[a]Numbers in parentheses are certified values. [b]Numbers in parentheses are reference values. [c]Method recovery (%) calculated as (sum of Hg species/total Hg) × 100.

using the $^{199}Hg/^{202}Hg$ ratios after correcting the counts for mass bias. For the SRM, a total Hg concentration of 31.9 ± 2.1 ng g^{-1} was found, which is in good agreement with the certified value (31.4 ± 1.7 ng g^{-1}) at 95% CL. The total Hg found in the blood samples ranged from 0.5−31 ng g^{-1} (Table 1, column 2).

Determination of Hg Species. The Hg species in the blood samples were determined by triple-spiking SIDMS as described in the experimental section. The spiked blood samples (with $^{199}Hg^{2+}$, $CH_3{}^{200}Hg^+$, and $C_2H_5{}^{201}Hg^+$) were analyzed by GC-ICP-MS after microwave-enhanced preparation and HS-SPME, and ion counts (cps) were recorded at m/z 199, 200, 201, and 202. SIDMS calculations were made using the mass bias corrected isotopic ratios of $^{199}Hg/^{202}Hg$, $^{200}Hg/^{202}Hg$ and $^{201}Hg/^{202}Hg$ for all the three species.

The species and concentrations of Hg found in the tested blood samples are presented in Table 1. The amounts of Hg^{2+} and CH_3Hg^+ found in the bovine blood SRM were in good agreement with the reference values at 95% CL.

All the three Hg species (i.e., Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$) were found in sample HB1, which was drawn 24 h after administering a thimerosal-containing vaccine to the subject. The presence of $C_2H_5Hg^+$ in the sample was apparent from the degradation of thimerosal, and its small fraction (1.7% related to total Hg) may be due to the very short half-life of the specie in blood.[2,3] Hg^{2+} and CH_3Hg^+ were found in samples HB2 and HB3 that were collected from individuals having no dietary restrictions. In samples HB1, HB2, and HB3, CH_3Hg^+ represented high fractions (>70%) of the total Hg, suggesting that the individuals were exposed to the specie through consumption of fish and/or other seafood products. The samples with the lowest concentrations of total Hg (HB4 and HB5) were found to exclusively contain inorganic Hg (Hg^{2+}). This result was in agreement with the fact that the subjects were not fish consumers.

The validity of the analytical results was evaluated using mass balance comparison. Mass balance validation, where the sum of the individual species equals the total elemental content in a

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sample, is one of the definitive means of validating speciated elemental concentrations. The total Hg determined in the blood samples (Table 1, column 2) was compared with the sum of the concentrations of Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$ found in the corresponding samples (Table 1, column 6). Statistical agreement was found between the two sets of measurements at 95% CL; the sum of the individual Hg species represented 100−111% of the total Hg in the samples.

Method-Induced Interconversions of Species. Interconversions between the species that may have occurred at any step beginning from the sample spiking up to the final analysis were tracked and corrected for by using the mathematical relationships in triple-spiking SIDMS. All the six possible interconversions of species stemming from methylation, demethylation, ethylation, and deethylation reactions (see Figure 3) were considered. The mathematically quantified method-induced transformations of species showed that the highest transformation was for deethylation of $C_2H_5Hg^+$ in sample HB1; close to 24% of the specie was converted to Hg^{2+}. Ethylation of Hg^{2+} to $C_2H_5Hg^+$ (0.3%) and demethylation of $C_2H_5Hg^+$ to CH_3Hg^+ (0.9%) were also identified in sample HB1. In all the tested samples, demethylation of CH_3Hg^+ to Hg^{2+} (1.2−15%) and methylation of Hg^{2+} to CH_3Hg^+ (0.1−3%) were tracked and corrected.

CONCLUSIONS

The study has demonstrated the use of microwave-enhanced solubilization and equilibration, HS-SPME, and GC-ICP-MS analysis in conjunction with triple-spiking SIDMS for accurate and reproducible quantitation of Hg^{2+}, CH_3Hg^+, and $C_2H_5Hg^+$ in human blood. The study describes the mathematical relationships in triple-spiking SIDMS and effectively shows its use for tracking and correcting for the transformation of Hg species resulting from method-induced methylation, ethylation, demethylation, and deethylation reactions. The distribution of the Hg species in the tested human blood samples clearly showed a direct relationship between the species and the subjects' source of exposure to Hg. The study demonstrated that the coupling of SIDMS with HS-SPME and GC-ICP-MS enables detection of Hg species existing in human blood at low parts per billion concentrations. The accuracy and reproducibility of the present method clearly demonstrated its superior capability in overcoming the commonly encountered imprecision in SPME. The present method is believed to be suitable for measuring the Hg species in other clinical samples.

ASSOCIATED CONTENT

Supporting Information

A pictorial presentation of the method is presented. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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