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Simultaneous Determination of Species-Specific Isotopic Composition of Hg by Gas Chromatography Coupled to Multicollector ICPMS

Vladimir N. Epov,*,† Pablo Rodriguez-Gonzalez,† Jeroen E. Sonke,‡ Emmanuel Tessier,† David Amouroux,† Laurence Maurice Bourgoin,‡ and Olivier F. X. Donard†

LCABIE, IPREM, CNRS UMR-5254, Pau University, 2 Avenue du President Angot, Pau, 64053, France, and LMTG UMR-5563 CNRS-IRD-OMP, University Paul Sabatier Toulouse 3, 14 Avenue Edouard Belin, Toulouse, 31400, France

This work presents the simultaneous online determination of the isotopic composition of different Hg species in a single sample by the hyphenation of gas chromatography (GC) with multicollector-inductively coupled plasma mass spectrometry (MC-ICPMS). With the use of commercially available instrumentation, precise and accurate species-specific Hg isotope δ values (per mil deviation of the Hg isotope ratio in the sample relative to a reference standard) have been obtained online from consecutive GC transient signals. The use of isothermal temperature programs to extend the elution of the Hg species, the proper selection of the peak integration window, as well as the preconcentration of real samples are critical to provide optimal counting statistics. Also, isotope ratio drift during transient signal elution was overcome by introducing a mixed Hg(II) and MeHg standard bracketing scheme and expressing all results using the δ-notation relative to SRM NIST-3133. Using the proposed methodology, we have obtained an external 2SD precision of 0.56 ‰ for δ^{202}Hg that is more than 10 times smaller than the overall Hg stable isotope variation thus far observed in terrestrial samples. The measurement of species-specific Hg isotopic composition relative to SRM NIST-3133 has been validated versus two other analytical techniques, i.e., conventional nebulization (CN) of Hg(II) solution and cold vapor (CV) generation of Hg^0 vapor. A good agreement between the species-specific δ values obtained by the different techniques has been obtained in secondary fractionated reference standard (UM-Almadén) and environmental matrixes, i.e., BCR-CRM 464 (tuna fish) and IAEA-085 (human hair). The results show mass-dependent and mass-independent fractionation in environmental samples, i.e., mass-independent fractionation of odd isotopes ^{199}Hg and ^{201}Hg in tuna fish was observed. This methodology provides new possibilities for the future study of species-specific stable isotope geochemistry of Hg and other trace metals.

During the last few decades, the biogeochemical cycle of mercury in the environment has received considerable attention. Mercury is considered to be a global pollutant due to the long average atmospheric residence time (0.8 years) of elemental gaseous Hg^0. Methylation in the aquatic environment produces the most toxic forms of Hg, such as methyl mercury (MeHg) and dimethyl mercury $(Me)_2Hg$.[1] Transfer of Hg within its biogeochemical cycle strongly depends on its speciation and can be summarized as follows: (i) volatilization from aquatic systems to the atmosphere as Hg^0, (ii) deposition from the atmosphere as Hg(II), (iii) methylation in the water domain, (iv) reduction to Hg^0, (v) accumulation in bottom sediments, (vi) bioaccumulation in the food chain, and (vii) diffusion and resuspension of the Hg species.[2] Mercury bioaccumulation is due to the greater trophic transfer efficiency of MeHg through the food chain. MeHg is able to enter and accumulate in the cytoplasm of phytoplankton cells rather than in the membrane,[2] and its interaction with biomolecules is not limited to S centers but also to C and N centers of DNA bases, pointing out additional mechanisms for the Hg mutagenicity.[3] MeHg is the most important species involved in the Hg human exposure via fish consumption, and its toxicity has led to the worldwide control and regulation of the Hg levels in foodstuffs.

Although Hg is one of the most studied trace elements in the environment, its biochemical cycle is not yet fully understood and further developments to understand the sources, reactivity, and bioaccumulation of the different Hg species in the environment are needed. In this sense, the study of the stable isotope geochemistry of Hg may provide a powerful tool to track the Hg cycle and pathways in the environment.[4] The measurement of stable Hg isotopic composition in different environmental samples has shown significant Hg isotope variations.[5–12] However, in spite of the evidence that the Hg mobility and bioaccumulation is

* To whom correspondence should be addressed. E-mail: vladimir.epov@univ-pau.fr. Fax: +33 559407781.

† ECABIE, IPREM, CNRS UMR-5254, Pau University.

‡ LMTG UMR-5563 CNRS-IRD-Universite Paul Sabatier Toulouse 3.

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extremely dependent on the chemical form (species) in which Hg is present in the environment, so far there is no methodology able to provide a simultaneous determination of the isotopic fractionation of different Hg species within the same environmental sample. Most of the previous studies report the total isotopic composition of Hg in the analyzed samples[5–7,11] whereas only a few of those report the species-specific isotopic fractionation of elemental mercury by its previous isolation using gold trap[8] or trapping solutions.[9] On the other hand, the species-specific isotopic fractionation of MeHg has been only reported in Certified Reference Materials containing 100% MeHg,[8,11] in real samples in which MeHg content is assumed to be 100%,[11] or by means of a tedious offline isolation of MeHg from the matrix by applying numerous sequential extraction steps.[12]

The study of species-specific isotopic fractionation requires precise and accurate analytical tools not only for the complete separation of such species but also for the isotope ratio measurement in different species. The hyphenation of inorganic mass spectrometry, i.e., inductively coupled plasma mass spectrometry (ICPMS), with a chromatographic technique is an advantageous approach for this type of study, particularly for Hg speciation.[13–15] With the use of hyphenated techniques such as liquid chromatography (LC) or gas chromatography (GC) coupled to ICPMS, the speciation analysis of a given sample can be performed online without applying any previous offline isolation of the species from the sample, which is not only a time-consuming step but also can lead to nonquantitative yield recoveries.

Besides, Hg isotope analysis by multicollector-ICPMS (MC-ICPMS) has gained great importance in identifying Hg sources and tracking Hg transformations in the environment.[9,16] In previous studies, it was demonstrated that the coupling of capillary GC and MC-ICPMS has opened a new door to the determination of chlorine,[17] bromine,[18] lead, and mercury[19] species-specific isotope ratios. However, the main limitation of this hyphenation for Hg was found to be the short time available for isotope ratio measurements, the small quantities of analytes introduced, and the pronounced isotope ratio drift during analyte transient passage. For this reason, the precision of the isotope ratio measurements obtained by GC-MC-ICPMS has been reported equal to 2−32‰, depending on the measured isotopic ratios,[19] which is 2−3 orders of magnitude higher than those obtained by measuring a continuous signal from conventional nebulization or cold vapor generation.[10]

The aim of this paper is the development of a novel approach for the precise online measurement of isotopic ratios and for the simultaneous determination of the isotopic fractionation of different Hg species in a single sample. These measurements are performed using the online hyphenation of GC with MC-ICPMS using commercially available instrumentation. The analytical strategy is based on (i) the extension of the chromatographic peak width by using isothermal temperature GC programs, (ii) an offline preconcentration step to obtain a larger signal and thus to improve the counting statistics for isotope ratio measurements, and (iii) a GC adapted sample−standard−sample bracketing scheme. The validation of the proposed methodology was performed by the comparison of the GC-MC-ICPMS isotope ratio measurement with those obtained using continuous sample introduction techniques such as conventional nebulization (CN) and cold vapor (CV) generation. Also, the applicability of this approach is demonstrated for some environmental and biological samples.

EXPERIMENTAL SECTION

Reagents. All solutions were prepared using ultrapure water (18 MΩ cm, Millipore). High-purity HNO_3 (J. T. Baker, Phillipsburg, NJ), ultrapure HCl (J. T. Baker), and H_2O_2 (J. T. Baker) were used throughout this work for the sample preparation. Standard reference material NIST-3133 (Mercury Standard Solution, 10 000 mg L^{-1}) was purchased from NIST (Gaithersburg, MD) and was used to prepare the test samples and δ-0 standard. A stock solution of MeHg (1 000 mg L^{-1} as Hg) was prepared from methylmercury chloride (Strem Chemicals, Inc., Bischheim, France) and was used for the preparation of fresh MeHg solutions as test samples and the bracketing standard. A stock solution of Tl (2664 mg L^{-1} in 5% HNO_3) was prepared from the standard reference material NIST-997 (Thallium Isotopic, NIST, Gaithersburg, MD) by dissolution in 8 M HNO_3. A buffer solution of pH = 4 was prepared using acetic acid (Sigma-Aldrich Lyon, France) and sodium acetate (Sigma-Aldrich). Sodium tetraethylborate ($NaBEt_4$) (purity 98%) and sodium tetrapropylborate ($NaBPr_4$) (purity 98%) were purchased from Galab (Geesthacht, Germany) and employed for the derivatization of the Hg species. All samples were extracted into hexane (Sigma-Aldrich) before the injection into the GC-MC-ICPMS. Two CRPG samples of inorganic mercury, named in this work as InHg-1 and InHg-2, having large positive and large negative mass-dependent Hg fractionation were obtained from the CRPG (Centre de Recherches Pétrographiques et Géochimiques, Nancy, France) and were used as in-house standards for the validation of the Hg isotope ratio measurements by GC-MC-ICPMS. Also, a stock solution of the secondary isotope reference material UM-Almadén was provided by the Department of Geological Science of the University of Michigan and used in this work for the validation of the Hg(II) GC-MC-ICPMS measurement. In addition, Hg isotopic ratios were measured in a Certified Reference Material BCR-CRM 464 (tuna fish) obtained from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) and in a Certified Reference Material IAEA-085 (human hair) obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). Tetramethyl ammonium hydroxide (TMAH) solution (Sigma-Aldrich) was used for the extraction of Hg species from fish samples.

Instrumentation. A Neptune MC-ICPMS instrument (Thermo Fisher Scientific, Bremen, Germany) located at the LMTG

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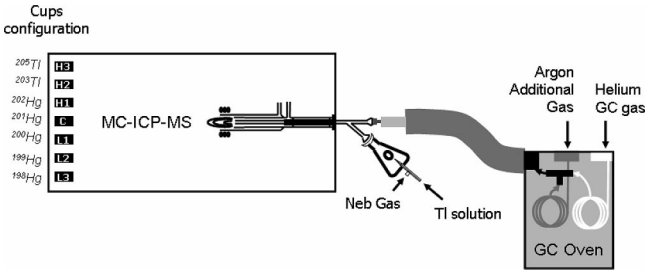

Figure 1. Schematic analytical setup for the measurement of species-specific Hg stable isotope ratios.

Table 1. Instrumental Parameters for the GC-MC-ICPMS Hyphenation

GC programs	ethylation	propylation
initial *T*, °C	36	44
initial time, min	7	7
ramp 1, °C/min	60	5
final T1, °C	53	45
hold time1, min	6	0
ramp 2, °C/min	60	60
final T2, °C	250	76
hold time 2, min		6
ramp 3, °C/min		60
final T3, °C		250

MC-ICPMS parameters	CN	CV	GC
rf power (W)	1100	1100	1100
auxiliary gas flow (L min^{-1})	0.63	0.63	0.63
coolant gas flow (L min^{-1})	15	15	15
nebulizer gas flow (L min^{-1})	1.1	1.1	1.1
carrier gas flow (L min^{-1})		0.10 (Ar)	0.25 (He)
mass resolving power	300	300	300
acquisition: blocks, cycles, integration time (s)	8, 7, 8	8, 7, 8	1, 600, 1

(Laboratoire des Mécanismes et Transferts en Géologie, Toulouse, France) was employed in this work for all the isotope ratio measurements. The instrument was equipped with a homemade double-inlet injector (see diagram presented in Figure 1) which allows the coupling of a gas chromatographic system Focus GC (Thermo Fisher Scientific, Milan, Italy) via the commercially available interface provided by this company. The GC was equipped with an automatic injector and a capillary column MXT (Silcosteel 30 m, i.d. 0.53 mm, and 1 μm coating). The Silcosteel capillary transfer-line was inserted directly into the injector inlet, whereas the additional injector inlet was used to connect an impact bead spray-chamber with a Meinhart nebulizer. This instrumental configuration enables the dual introduction of gaseous samples and isotopically certified Tl standard solution for the mass-bias correction. The MC-ICPMS instrumental parameters, including the GC temperature programs, are presented in Table 1. Helium was employed as a carrier gas at 25 mL min^{-1} at constant flow, and a sample volume of 2 μL was injected using, in all cases, a split/splitless injector with 1 min splitless time at 250 °C.

An oven (model UM/SM 100, Memmert, Schwabach, Germany) was used to perform the digestion of the IAEA-085 human hair standard. The preconcentration of the derivatized extracts was carried out under a gentle stream of argon using a Mini-Vap Evaporator/Concentrator from Supelco (Sigma-Aldrich). The total digestion of the samples was carried out using a DigiPREP digestion system (SCP-Science, Courtaboeuf, France). For validation purposes, cold vapor (CV) generation MC-ICPMS and conventional nebulization (CN) were employed as complementary techniques to determine the total Hg isotopic composition of NIST-3133, MeHg Strem, CRM-464, IAEA-085, InHg-1, InHg-2, and UM-Almadén. A Perkin-Elmer cold vapor generator for the introduction of Hg^0 and an Aridus II desolvation system (CETAC) for the introduction of SRM 997 thallium were used for CV-MC-ICPMS (operating conditions are shown in Table 1). Hg(II) in samples and standards was reduced online in the cold vapor generator using 3% w/v $SnCl_2$ in 1 N HCl. Typical instrumental sensitivity was 2 V on ^{202}Hg for 25 ng g^{-1} Hg introduced at 0.3 mL min^{-1}. When CN is used, an impact-bead spray chamber and a Meinhart nebulzier were used with an approximate 50-fold lower sensitivity than for CV. A wash-out time for both CV and CN of 12 min was required to ensure that the blank level was <1% of the preceding sample or standard signal. Gain and baseline calibration was performed on a daily basis.

The Russell equation was used for mass-bias correction of Hg isotopic ratios (see eq 1):

$$\left(\frac{^{xxx}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_{\mathrm{true}} = \frac{\left(\frac{^{xxx}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_{\mathrm{measured}}}{\left(\frac{xxx}{197.966743}\right)^{f}} \quad (1)$$

where *xxx* is the mass of the Hg isotope for which the isotopic ratio is calculated, i.e., 201.970 617 for ^{202}Hg, 200.970 277 for ^{201}Hg, 199.968 300 for ^{200}Hg, and 198.968 254 for ^{199}Hg; *f* was calculated using measured isotopic ratios for Tl and the Russell correlation:

$$f = \log_{1.009864}\left(\frac{\left(\frac{^{205}\mathrm{Tl}}{^{203}\mathrm{Tl}}\right)_{\mathrm{measured}}}{2.38714}\right) \quad (2)$$

The standard−sample−standard bracketing mode (concentrations and hence sensitivities between samples and standards were matched within 10%), with NIST-3133 and MeHg Strem as bracketing standards for Hg(II) and MeHg species, respectively, was used to calculate δ values:

$$\delta^{xxx}\mathrm{Hg} = \left(\frac{\left(\frac{^{xxx}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_{\mathrm{sample}}}{\left(\frac{^{xxx}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_{\mathrm{std}}} - 1\right) \times 1000 \quad (3)$$

where $(^{xxx}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{std}}$ refers to the average isotope ratio of Hg(II) or MeHg species measured before and after the sample. Isotopes ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{203}Tl, and ^{205}Tl were detected on Faraday cups L3, L2, L1, C, H1, H2, and H3, respectively, for all CV-, CN-, and GC-MC-ICPMS setups. In addition, "capital delta" (Δ^{xxx}Hg), the deviation from mass dependency (in ‰) was calculated using eq 4:

$$\Delta^{xxx}\mathrm{Hg} = 1000(\{\ln[(\delta^{xxx}\mathrm{Hg}/1000) + 1]\} - k\{\ln[(\delta^{202}\mathrm{Hg}/1000) + 1]\}) \quad (4)$$

where *k* is the kinetic scale factors, which are dependent on the calculated isotope, 0.2520 for ^{199}Hg, 0.5024 for ^{200}Hg, and 0.7520

for ^{201}Hg. Detailed description of eq 4 for the Hg isotopes is presented by Blum and Bergquist.[10]

All confidence intervals in this paper are calculated as 2 times the standard deviation (2SD).

Sample Preparation Procedures. *Total Digestion of the Samples.* The validation of the species-specific Hg stable isotopic composition measurements by GC-MC-ICPMS was carried out by comparing the obtained δ values in samples containing MeHg with those obtained using CN-MC-ICPMS and CV-MC-ICPMS. For this purpose, 0.5 g or 0.5 mL of the samples containing MeHg, i.e., MeHg Strem (99.999% of MeHg), CRM-464 (98% of MeHg), and IAEA-085 (92% of MeHg), were mixed with 5 mL of HNO_3 and left overnight. Then, 2.5 mL of H_2O_2 was added, and this mixture was heated in a digiPREP system at 80 °C for 4 h (90 min of ramp and 2.5 h of heating time) such that all the Hg was 100% converted to Hg(II).

Extraction of the Hg Species from Tuna Fish and Human Hair. A sample size of 0.05−0.3 g of the tuna fish CRM-464 was mixed with 4 mL of 25% TMAH solution[20] in a 22 mL vial with a screw cap. Then, the sample was left at room temperature (20 °C) for 3 h to ensure total digestion.[21] When the human hair sample IAEA-085 was analyzed, 0.02 g of the CRM was mixed with 5 mL of 6 N HNO_3 in a 22 mL vial with a screw cap. Then, the sample was introduced to an oven at 70 °C for 2 h for the digestion.

Derivatization of the Samples for the GC-MC-ICPMS Isotope Ratio Measurement. Ethylation or propylation of the Hg species was carried out in 15 mL glass vials in which a volume of 4 mL of acetic acid/sodium acetate buffer (0.1 mol L^{-1}) was mixed with an appropriate amount of the Hg standard solution or the extracted sample. Then the pH was readjusted to pH 4 with ultrapure hydrochloric acid or with ammonium hydroxide, and the mercury species were derivatized and extracted into an organic solvent during 5 min of manual shaking after the addition of 0.5 mL of 1% w/v of sodium tetraethylborate or tetrapropylborate and 2 mL of hexane. Finally, the organic phase was transferred to a 2 mL chromatographic vial and stored at −18 °C. Before the isotope ratio measurement, the samples were preconcentrated when necessary under a gentle stream of argon to obtain a final concentration of the derivatized Hg species in the organic phase higher or equal to 200−250 ng Hg g^{-1}.

Derivatization of the Samples for GC-Q-ICPMS Isotope Dilution Analysis. The determination of the MeHg concentration in the certified reference materials IAEA-085 (human hair) and CRM-464 (tuna fish) was carried out by species-specific isotope dilution analysis in order to test the efficiency of the digestion procedures employed. For this purpose, 0.05 g of the extract was mixed in a vial containing buffer solution with an appropriate amount of a 10 ng g^{-1} solution of ^{201}Hg-enriched MeHg according to the random error propagation theory[22] (0.08 and 0.150 g for IAEA-085 and CRM-464, respectively). The results obtained in both samples are shown in Table 2. As can be observed, the proposed procedures provide a quantitative digestion of the Hg species without promoting the degradation of the MeHg in the samples as demonstrated for three independent analyses of each CRM.

Table 2. MeHg Concentrations Obtained for Three Independent Analyses of the CRMs: IAEA-085 and BCR-CRM 464

extraction replicate	concentration (μg Hg g^{-1})	RSD (%)
	IAEA-085, Human Hair	
1	21.7 ± 1.3	5.8
2	21.0 ± 0.2	1.1
3	22.4 ± 0.6	2.5
mean	21.7 ± 0.5	2.4
certified value	21.3 ± 0.9	4.4
	BCR-CRM 464, Tuna Fish	
1	5.02 ± 0.19	3.9
2	5.06 ± 0.06	1.3
3	4.99 ± 0.04	0.9
mean	5.03 ± 0.04	0.7
certified value	5.12 ± 0.16	3.1

RESULTS AND DISCUSSION

Optimization of the Measurement. Previous studies have already described in detail the hyphenation of GC to MC-ICPMS for the measurement of species-specific isotope ratios of Hg,[19,23] Pb,[19,24,25] Sb,[26] Cl,[17] and Br.[18] However, the main limitation in these works was found to be the very short transient signal (2−5 s) provided by GC, which does not allow the measurement of isotope ratios with the high precision required for environmental studies. These previous studies demonstrated that either the integration of the chromatographic peak or the "point-by-point" measurement of the isotope ratio during the peak profile was hardly able to provide an internal precision better than 1‰ (1SD). In this work we aimed to achieve a 2SD external reproducibility <0.7‰ for δ^{202}Hg on ∼500 pg Hg injections, the latter reflecting the amount of total extractable Hg species from moderately concentrated (200 ng Hg g^{-1}) environmental samples. Note that 0.7‰ 2SD is roughly 10 times smaller than the overall fractionation of Hg isotopes encountered thus far in environmental samples (∼7‰), and 2 times larger than the counting statistics limited uncertainty on δ^{202}Hg of 0.35‰ for a 500 pg analysis.

Optimization of the Gas Chromatographic Separation. An improvement of the ion counting statistics during the transient signal was achieved by several modifications in comparison with a previous study.[19] First, isothermal temperature programs (Table 1) were employed to increase the chromatographic peak width of the derivatized species to at least 30−60 s for both Hg(II) and MeHg. This is approximately 10 times larger than those obtained by conventional GC-ICPMS operating conditions. For this purpose, the temperature programs were first individually optimized for Hg(II) and MeHg, and then both programs were combined and optimized for the simultaneous measurement of the Hg isotope ratios in both ethylated and propylated species (Table 1). A GC-MC-ICPMS chromatogram of a standard containing 250 ng Hg g^{-1} of both Hg(II) NIST-3133 and MeHg Strem is presented in

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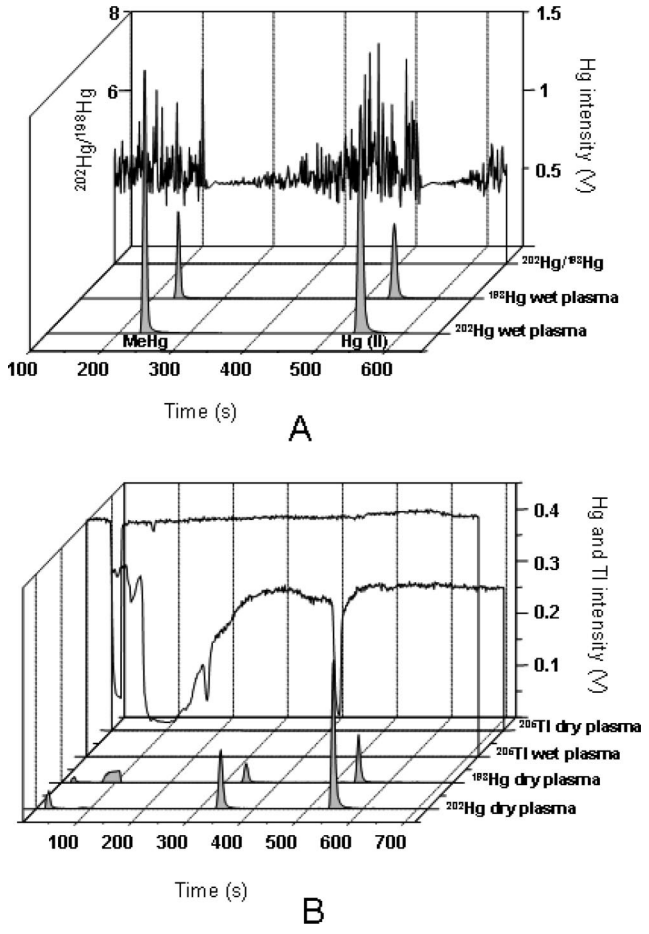

Figure 2. (A) GC-MC-ICPMS chromatogram of a standard containing 250 ng Hg g^{-1} of MeHg and Hg(II) and the resulting $^{202}Hg/^{198}Hg$ isotope ratios (B) ^{205}Tl signal obtained during a GC-MC-ICPMS chromatogram of a standard containing 100 ng Hg g^{-1} of MeHg and Hg(II) in wet (impact bead spray-chamber equipped with the Meinhard nebulizer) and dry-plasma conditions (desolvating unit).

Figure 2A. From this figure we can observe an efficient chromatographic resolution of Hg species and two stability areas for the isotope ratios located within the development of MeHg and Hg(II) chromatographic peak profiles. With the use of an integration time of 1 s, 30−50 points can be used for each species to calculate the isotope ratio of the transient signals.

Optimization of the Mass-Bias Correction Procedure. Instrumental mass-bias was corrected using eqs (1) and (2). The introduction of the Tl solution into the ICP (Figure 1) for the correction of the mass-bias was also optimized by comparing two sample introduction systems: a desolvating unit Aridus with a microconcentric nebulizer and an impact bead spray-chamber equipped with a Meinhart nebulizer. Figure 2B shows the profiles of the Tl signal obtained using the two mentioned sample introduction systems during the GC-MC-ICPMS chromatogram of the same standard solution containing MeHg and Hg(II). Even though the desolvating unit was normally more sensitive and requires less amount of Tl to be introduced, the Tl signal obtained was found to be highly unstable, due to the disturbances in the plasma. This can be explained by the fact that the desolvating unit in combination with GC produces a "dryer" plasma than when using a spray-chamber equipped with a Meinhart nebulizer. Therefore, when using the desolvation,

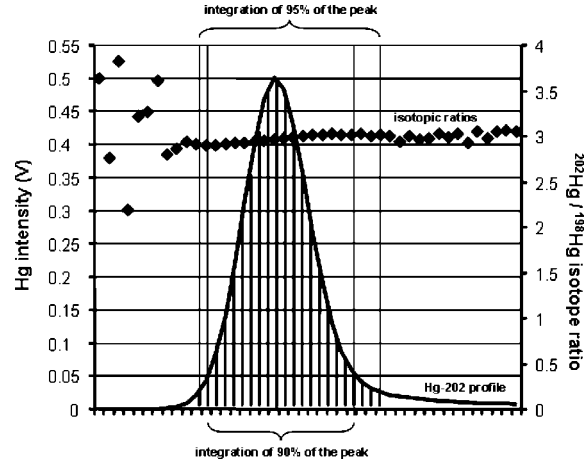

Figure 3. Integration of 90% and 95% of the peak area of the chromatographic peak of a 200 ng g^{-1} standard of Hg(II) and the resulting $^{202}Hg/^{198}Hg$ isotope ratios within these areas.

the level of oxygen atoms in the plasma was not enough to rapidly oxidize the organic solvent (2 μL of hexane) and the different eluting organic compounds during the GC run. However, if Tl is introduced using the impact bead spray-chamber, only a short initial disturbance in the plasma appears during the elution of the hexane and does not overlap with the elution of the analytes. Hence, oxygen atoms introduced as water into the plasma are found to be helpful to oxidize the organic carbon during a short time. This explanation was confirmed by the resulting carbon deposition on the cones when using the desolvating unit and the absence of such deposition when using the impact bead spray-chamber equipped with the Meinhard nebulizer. Moreover, Figure 2B demonstrates that during elution of the organic solvent under dry-plasma conditions, signals at m/z's = 198 and 202 are significantly different from the natural abundances of corresponding Hg isotopes. This means that unburned molecules of the solvent may create molecular clusters with the same m/z's as Hg isotopes whereas when using wet-plasma conditions this is not the case. According to these results, the conventional ICPMS spray-chamber was used in all the experiments for the Tl introduction, rather than desolvating units.

Data Treatment, Integration Window. An important part during the optimization of the analytical method was the proper processing of the obtained data to get precise isotope ratios. In this work, we have compared different calculation approaches of the species-specific isotope ratios obtained from the GC transient signals. The results were calculated either by integration of the different parts of the peak area or by averaging the isotope ratios calculated for each point corrected for the background throughout the whole chromatographic peak ("point-by-point" or "p-b-p" approach). Figure 3 shows, as an example, the integrated peak areas, with the use of the points that have intensity higher than 90% and 95% of the peak height for Hg(II). These areas were referred to as 90% and 95% of the peak, respectively. Only the points which have intensity higher than 10% of the maximum peak height were selected for the calculation of isotopic ratios using 90% of the peak, and the points with the intensity higher than 5% of the maximum peak height were taken into account when using 95% of the peak.

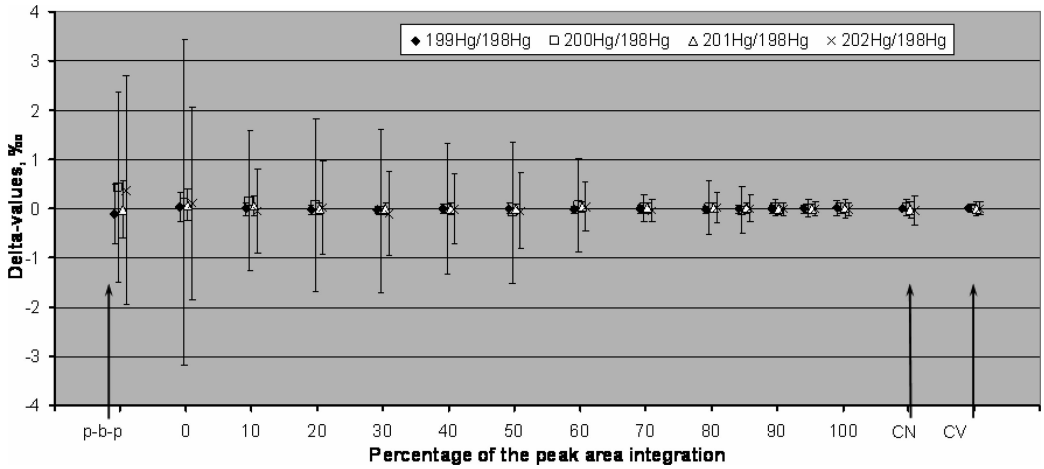

Figure 4. Precision of the δ values of the standard reference material NIST-3133 bracketed with NIST-3133 using GC-MC-ICPMS. Increasing integration percentages of the chromatographic peak are compared with the "point-by-point" calculation and the CN-MC-ICPMS and CV-MC-ICPMS measurements.

Thus, the points located on the right and left shoulders of the GC peak which were lower than the corresponding minimum percentages were rejected. Accordingly, the integration of 0% of the peak represents the calculation of isotope ratios using just one point at the maximum intensity of the peak.

Figure 4 compares the mean and 2SD of δ values for the standard reference material NIST-3133 bracketed with NIST-3133 using GC-MC-ICPMS, CN-MC-ICPMS, and CV-MC-ICPMS. In all cases, the δ values for each sample introduction technique were calculated using eqs 1−3. For the case of GC-MC-ICPMS, the average δ values for 38 bracketing replicates were calculated by integrating different percentages of the chromatographic peak. The results obtained using the "point-by-point" approach were calculated for each point within the peak, rejecting the ratios outside of the confidence interval of ±2SD. The average δ^{202}Hg obtained using CN-MC-ICPMS and CV-MC-ICPMS are −0.04 ‰ ± 0.59 ‰ and 0.01 ‰ ± 0.28 ‰, respectively. The precision of the δ values using different approaches is shown as 2SD error bars. As can be observed, the best precision was obtained when applying integration of 90, 95, and 100% of the chromatographic peak whereas the worse precision was obtained using the "point-by-point" calculation approach or using one point at the peak maximum (0% of integration).

According to these results, the integration of 95% of the peak was selected for further experiments, as the establishment of the start and the end of the transient signal was easier and more reproducible than when integrating 100% of the peak.

Analytical and Data Reduction Strategy. The analytical and data reduction scheme for the precise species-specific Hg isotopic composition measurement of environmental samples is presented in Figure 5. Central to the data reduction scheme is the adoption of the standard−sample−standard bracketing measurement sequence and the use of the δ notation to express species-specific isotopic compositions relative to the δ-0 reference standard NIST-3133.[10] Since Hg(II) and MeHg are eluted at different times and therefore under different conditions, these species should ideally be bracketed by the same species in the mixed bracketing standard and use the same GC-temperature program. The NIST-3133 Hg(II) standard was chosen as the bracketing species for sample Hg(II), and the commercial

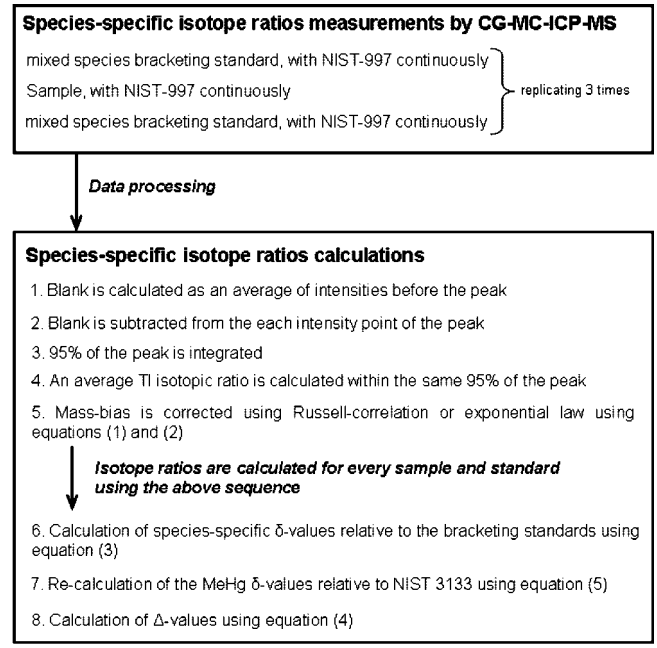

Figure 5. Species-specific sample−standard−sample bracketing and data reduction schemes for the measurement of species-specific Hg isotopic ratios.

Strem MeHg standard was chosen to bracket sample MeHg. The sample δ^{202}MeHg expressed relative to Strem is subsequently recalculated on the NIST-3133 scale by correcting for the isotopic difference between NIST-3133 and Strem MeHg. Strem MeHg was therefore characterized by CN-MC-ICPMS and CV-MC-ICPMS vs NIST-3133 and found to be enriched in the lighter isotopes by −0.50 ‰ ± 0.28 for δ^{202}Hg (2SD, n = 13). Simultaneously with the chromatographic introduction of the Hg analytes, Tl is nebulized into the ICP for the correction of the instrumental mass-bias (Figure 1). The data processing (Figure 5) involves a blank subtraction from each intensity point of the peak, calculation of the average isotopic ratio within the 95% of the peak (Figure 3), mass-bias correction using the Russell correlation (eqs 1 and 2), and calculation of δ^{xxx}Hg by the bracketing of Hg(II) and MeHg in the sample with NIST-

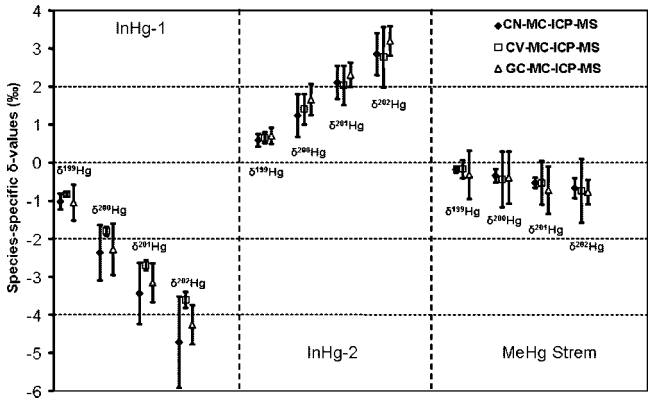

Figure 6. δ values obtained for the CRPG samples InHg-1, InHg-2, and an oxidized MeHg Strem standard by GC-MC-ICPMS (n = 12−21), CN-MC-ICPMS, and CV-MC-ICPMS (n = 6−9).

3133 and Strem MeHg in the standard, respectively (eq 3). The conversion of the δ values from MeHg Strem to NIST-3133 is calculated using eq 5:

$$(\delta^{xxx}\mathrm{Hg})_{\mathrm{sample-NIST\text{-}3133}} = \left[\left(\frac{(\delta^{xxx}\mathrm{Hg})_{\mathrm{MeHgSTREM-NIST\text{-}3133}}}{10^3} + 1\right)\left(\frac{(\delta^{xxx}\mathrm{Hg})_{\mathrm{sample-MeHgSTREM}}}{10^3} + 1\right) - 1\right] \times 10^3 \quad (5)$$

where $(\delta^{xxx}\mathrm{Hg})_{\mathrm{MeHgSTREM-NIST\text{-}3133}}$ is the δ value of MeHg Strem calculated from NIST-3133 using the average value obtained by cold vapor and continuous nebulization and $(\delta^{xxx}\mathrm{Hg})_{\mathrm{sample-MeHgSTREM}}$ is the δ value of the MeHg in the sample calculated vs the MeHg Strem by GC-MC-ICPMS. Mass-dependency was estimated by the calculation of Δ^{xxx}Hg using eq 4.

Validation of the Developed Technique. GC-MC-ICPMS validation was carried out by comparing the precision and accuracy of the absolute isotope ratios and δ values of InHg-1, InHg-2, and Strem MeHg. The Hg isotopic composition of the samples and reference materials was first characterized by CN- and CV-MC-ICPMS. For the calculation of Hg(II) δ values, we used the fractionated InHg-1 and InHg-2 in-house standards, which have large negative and positive δ values of δ^{202}Hg of −3.55 ‰ and +2.56 ‰, respectively.[27] For the calculation of the MeHg δ values, we used a MeHg Strem standard which was previously oxidized to Hg(II) with 100% yield recovery in order to carry out the CV-MC-ICPMS and CN-MC-ICPMS measurements. In this way, all the standards were bracketed with NIST-3133 and measured 6−9 times by each of the different techniques over the course of 1 year. Figure 6 shows that both for Hg(II) and MeHg species a good agreement between the δ values obtained by the three different approaches was found for every sample and for all the isotope ratios (Figure 6). The GC-MC-ICPMS long-term external reproducibility (2SD) for δ^{202}Hg of these materials is therefore 0.45 ‰ (n = 12) and 0.32 ‰ (n = 21) for Hg(II) and MeHg, respectively.

Preconcentration of the Hg Species Prior to Isotope Ratios Measurements. Because of the low level of Hg species in real samples, conventional sample preparation procedures for GC-Q-ICPMS do not usually provide sufficient ion counting statistics for the precise measurement of isotope ratios by this technique. From the previous optimizations, we found that a species concentration level in the final organic solvent (hexane) of 200−250 ng Hg g^{-1} provided a transient signal of approximately 0.5−1 V (Figures 2 and 3) at the top of the peak leading to a good precision in the isotope ratios (see Figure 6). However, if we assume an expected MeHg or Hg(II) concentration range in real samples of 200−1000 ng Hg g^{-1} (dry weight) a 10−50-fold preconcentration is required using our sample preparation protocols. Because of its simplicity, and in spite of the risk of volatilization of the Hg species, an evaporative preconcentration of Hg in the organic phase was tested. Hg isotopic fractionation during hexane evaporation and the associated potential loss of the Hg species was carefully evaluated before its final application. For this purpose, an initial solution of either NIST-3133 Hg(II) or Strem MeHg of 250 ng g^{-1} in the organic phase was diluted 25 times in hexane and subsequently preconcentrated 25 times by evaporation under a gentle stream of Ar. Then three independent evaporation replicates were performed for both ethylated and propylated species, and isotopic ratios in these samples were measured by GC-MC-ICPMS. The δ values of the preconcentrated Hg species were calculated by the bracketing of the evaporated samples with the corresponding nonevaporated standards, and the results obtained are presented in Table 3. As can be observed, no isotopic fractionation was detected for Hg(II) and MeHg when using either propylation or ethylation for the derivatization followed by the evaporation. Therefore, the proposed preconcentration procedure was selected as it can be used for a wider range of Hg concentration in environmental samples.

Table 3. δ Values (‰) Measured for Hg Isotopic Ratios in Evaporated vs Nonevaporated Samples

ethylated species	Hg(II)	MeHg
δ^{199}Hg	−0.15 ± 0.60	−0.07 ± 0.70
δ^{200}Hg	−0.05 ± 0.90	0.07 ± 0.06
δ^{201}Hg	0.09 ± 0.10	−0.18 ± 0.28
δ^{202}Hg	−0.08 ± 0.66	0.10 ± 0.44
propylated species	**Hg(II)**	**MeHg**
δ^{199}Hg	0.05 ± 0.50	−0.09 ± 0.34
δ^{200}Hg	−0.12 ± 0.36	−0.07 ± 0.36
δ^{201}Hg	0.10 ± 0.10	0.13 ± 0.98
δ^{202}Hg	−0.09 ± 0.32	−0.09 ± 0.54

Choice of the Bracketing Standards. The NIST-3133 (Mercury Standard Solution) has been recently proposed[10] as the Hg δ-0 bracketing standard. Since this standard contains 100% of Hg(II), we are using NIST-3133 for the bracketing of Hg(II) in the samples measured by GC-MC-ICPMS. However, the measurement of δ values for MeHg requires the use of a bracketing standard containing 100% of MeHg.

In this work we have attempted first to synthesize MeHg from NIST-3133 using a methylation protocol described elsewhere,[28] and the δ values for the synthesized MeHg were measured vs initial NIST-3133 δ-0 standard using GC-MC-ICPMS. As observed

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Table 4. δ Values for Different MeHg Standards vs NIST-3133

	n	δ^{199}Hg	δ^{200}Hg	δ^{201}Hg	δ^{202}Hg	Δ^{199}Hg	Δ^{200}Hg	Δ^{201}Hg
MeHg produced from NIST-3133	3	−0.34 ± 0.65	−0.82 ± 0.91	−1.00 ± 0.37	−1.33 ± 0.90	−0.01 ± 0.59	−0.15 ± 0.54	−0.00 ± 0.46
MeHg Strem	13	−0.11 ± 0.13	−0.27 ± 0.14	−0.37 ± 0.24	−0.50 ± 0.29	0.01 ± 0.06	−0.02 ± 0.08	−0.01 ± 0.06

Table 5. Species-Specific δ Values (‰) for Environmental Reference Materials Relative to NIST-3133

	n	δ^{199}Hg	δ^{200}Hg	δ^{201}Hg	δ^{202}Hg	Δ^{199}Hg	Δ^{200}Hg	Δ^{201}Hg
UM-Almadén Mine (Spain)								
GC-MC-ICPMS (in Hg(II) species)	3	−0.23 ± 0.11	−0.28 ± 0.19	−0.50 ± 0.23	−0.61 ± 0.12	−0.08 ± 0.14	0.03 ± 0.20	−0.04 ± 0.31
CV-MC-ICPMS (in total Hg)	34	−0.14 ± 0.08	−0.23 ± 0.13	−0.42 ± 0.15	−0.51 ± 0.17	−0.01 ± 0.08	0.02 ± 0.08	−0.04 ± 0.07
Blum and Bergquist[6]		−0.14 ± 0.06	−0.27 ± 0.04	−0.44 ± 0.07	−0.54 ± 0.08			
BCR-CRM 464, Tuna Fish (Total Hg 5.24 ± 0.10 mg Hg kg^{-1})								
GC-MC-ICPMS (nonevaporated)	3	2.44 ± 0.87	0.61 ± 0.54	2.85 ± 0.51	0.93 ± 0.67	2.21 ± 0.70	0.14 ± 0.24	2.15 ± 0.07
GC-MC-ICPMS (evaporated)	3	2.69 ± 0.43	0.38 ± 0.36	2.61 ± 0.48	0.65 ± 0.30	2.52 ± 0.44	0.05 ± 0.46	2.11 ± 0.46
CV-MC-ICPMS (in total Hg)	7	2.33 ± 0.11	0.37 ± 0.14	2.23 ± 0.18	0.59 ± 0.20	2.18 ± 0.08	0.07 ± 0.08	1.79 ± 0.08
IAEA-085, Human Hair (Total Hg 23.2 ± 0.8 mg Hg kg^{-1})								
GC-MC-ICPMS (nonevaporated)	3	−0.20 ± 0.74	−0.27 ± 0.48	−0.44 ± 0.43	−0.53 ± 0.87	−0.12 ± 0.57	−0.10 ± 0.24	−0.02 ± 0.16
GC-MC-ICPMS (evaporated)	3	−0.21 ± 0.47	−0.34 ± 0.63	−0.38 ± 0.34	−0.37 ± 0.41	−0.27 ± 0.38	0.00 ± 0.54	−0.14 ± 0.60
CV-MC-ICPMS (in total Hg)	6	−0.12 ± 0.07	−0.18 ± 0.06	−0.31 ± 0.15	−0.37 ± 0.15	−0.02 ± 0.04	0.01 ± 0.03	−0.03 ± 0.06

in Table 4, the chemical methylation of NIST-3133 induced an unpredictable mass dependent isotopic fractionation which rules out its use as the MeHg isotope reference standard. Our previous results obtained for abiotic methylation demonstrate mass-dependent enrichment of the product (MeHg) with lighter isotopes (Table 4). This also suggests that the recovery yield of abiotic methylation was not 100%, and we expect it is dependent on the experimental conditions. Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg values (Table 4) for synthesized MeHg prove the absence of mass-independent fractionation during the synthesis.

Since during the methylation of NIST-3133 the isotopic ratios are changed due to the fractionation, we decided to use commercially available MeHg Strem standard for the bracketing of MeHg species. Hence, we measured the δ values for MeHg Strem versus NIST-3133 using both CN- and CV-MC-ICPMS. For this purpose, MeHg was digested and converted to Hg(II) using HNO_3 and H_2O_2 (see Experimental Section) .The recovery for the total MeHg Strem digestion was found to be 102.1 ± 3.6% (1SD). δ values for MeHg Strem measured by different techniques can be found in Figure 6. The mean δ values obtained using both CN- and CV-MC-ICPMS are presented in Table 4. We observed isotopic mass-dependency for MeHg Strem, which was proven by the values of Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg (Table 4). Following the measurement of isotopic ratios for MeHg in the sample vs MeHg Strem by GC-MC-ICPMS, δ values for MeHg in the sample is recalculated vs NIST-3133 using the values presented in Table 4 and eq 5.

In addition, we have observed the absence of any degradation of Hg(II) and MeHg mixed standards kept in hexane for 5 days in the refrigerator between the analysis. Hence, the isotopic composition of these species in the mixture was stable during this time. Technically, future GC-MC-ICPMS studies by different laboratories can use any Hg(II) and MeHg standard to make up the mixed species bracketing standard, as long as their isotopic composition has been calibrated against NIST-3133. However, there is a substantial gain in precision and intercomparability if all laboratories use the same Hg(II) and MeHg standards. In line with a recent recommendation,[10] we suggest NIST-3133 as the reference standard for Hg(II). Until a MeHg reference standard with a guaranteed isotopic homogeneity and stability comes available, each laboratory will need to calibrate its own MeHg standard relative to NIST-3133.

Validation with Environmental Samples. After the development of the analytical methodology, we performed GC-MC-ICPMS measurements of Hg isotope ratios in three different environmental samples: BCR-CRM-464 (tuna fish), IAEA-085 (human hair), and secondary reference standard UM-Almadén (made from Hg mined from Almadén, Spain, the largest industrial Hg source in the world). The first two samples contain predominantly MeHg (98% and 92%, respectively), while the third one contains only inorganic Hg(II) species. The concentration of MeHg in biological samples is presented in Table 2, and total Hg concentrations for these samples are displayed in Table 5.

For GC-MC-ICPMS measurement of Hg(II) species in environmental samples, the direct bracketing by NIST-3133 was used. The UM-Almadén sample serves to intercompare the δ values obtained by GC-MC-ICPMS for Hg(II) not only with another sample introduction technique (CV) but also with previous results obtained by other laboratories.[10] As can be observed in Table 5, the δ values obtained in our laboratory by GC-MC-ICPMS (δ^{202}Hg = −0.61 ± 0.12‰) and CV-MC-ICPMS (δ^{202}Hg = −0.51 ± 0.17‰) are in good agreement with those reported by Blum and Bergquist (δ^{202}Hg = −0.54 ± 0.08‰).[10] Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg (Table 5) demonstrate mass dependency for the UM-Almadén sample.

The validation of the species-specific MeHg δ values was carried out by comparing the values obtained by GC-MC-ICPMS with those obtained using CV-MC-ICPMS in environmental matrixes. For the CV-MC-ICPMS analyses, those samples were previously digested in order to convert the endogenous MeHg into Hg(II) (see Experimental Section). The species-specific δ values for MeHg were measured by GC-MC-ICPMS vs MeHg Strem, followed by the recalculation of the results versus NIST-3133 using eq 5. CN-MC-ICPMS was not used, since this technique is not suitable for the analysis of complex matrixes containing high concentrations of other major elements or compounds.

For BCR-464 (tuna fish), mass-dependent fractionation (MDF) for even isotopes ^{198}Hg, ^{200}Hg, and ^{202}Hg and strong mass-

independent fractionation (MIF) for odd isotopes ^{199}Hg and ^{201}Hg was observed. MIF anomalies, Δ^{199}Hg and Δ^{201}Hg, which measure the deviation of $^{199/198}$Hg and $^{201/198}$Hg ratios relative to the equilibrium mass fractionation line with the $^{202/198}$Hg amount equal to +2.16‰, +1.77‰ for CV-MC-ICPMS and +2.37‰, +2.13‰ for GC-MC-ICPMS measurements. The ratio of MIF anomalies, (Δ^{199}Hg/Δ^{201}Hg) is equal to 1.22 (for CV-MC-ICPMS) and 1.11 (for GC-MC-ICPMS), suggesting that nuclear spin fractionation,[29] rather than nuclear volume fractionation,[30] is the MIF mechanism involved and that potentially aquatic photoreduction is the responsible process.[11]

Mass-dependent fractionation of all Hg isotopes was observed for the IAEA-085 (human hair) standard (Table 5). The last was proven by Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg values presented in Table 5. Since IAEA-085 is an SRM that was spiked (by the IAEA) with 98.7% exogenous MeHg, its measured isotopic composition essentially reflects that of the spiked material. It is interesting to note that this isotopic composition is similar to the MeHg Strem isotopic composition (Table 4). Table 5 shows that GC-MC-ICPMS and CV-MC-ICPMS yield similar results for both biological SRMs.

In addition, the influence of the preconcentration step was also studied for the analysis of less concentrated samples. Both CRM-464 (tuna fish containing around 5 μg g^{-1}) and IAEA-085 (human hair containing around 25 μg g^{-1}) were analyzed by derivatizing three independent extraction replicates, each containing 10 and 500 ng of MeHg and extracted into 2 mL of hexane. The samples containing 5 ng g^{-1} in the organic phase were 50 times preconcentrated as described in the Experimental Section in order to reach 250 ng g^{-1}. When working with real matrixes, ethylation was selected in all cases as the derivatization procedure due to the observed better stability of the reagent and better derivatization efficiency by $NaBEt_4$ compared to $NaBPr_4$.[31] The results presented in Table 5 demonstrate a good accuracy of the results for the measurement of δ values in different Hg species for both biological samples using GC-MC-ICPMS. Also, the nonpreconcentrated and preconcentrated samples using evaporation of the organic phase under Ar provided same species-specific δ values, meaning that the proposed preconcentration step can be used for the analysis of environmental samples when necessary.

CONCLUSIONS

This work reports the first analytical methodology capable of determinating the species-specific stable isotopic composition of Hg within the same sample. We have demonstrated that using commercially available instrumentation, precise and accurate species-specific Hg isotope δ values can be obtained online from different GC transient signals. The use of isothermal temperature programs to extend the elution time of the analytes, the proper selection of the integrated peak area window as well as the preconcentration of real samples to provide optimal counting statistics are critical to obtain an average external 2SD precision of 0.56 ‰, that is about 12 times smaller than the overall Hg stable isotope variation thus far observed in terrestrial samples. The optimization and validation of the GC-MC-ICPMS measurements was successfully carried out on different standards and real samples by the comparison with previously published results as well as with other complementary techniques providing continuous signals such as CN-MC-ICPMS and CV-MC-ICPMS.

The reference standard NIST-3133 is proposed in this work to be used as bracketing and δ-0 standard for Hg(II) species. For MeHg species, a universally accepted MeHg reference standard that is isotopically homogeneous and stable is required but not yet available. Commercial MeHg standards that have been calibrated against NIST-3133 are the only current alternative. MeHg Strem was used in this study as a bracketing standard for MeHg species. Since Hg mobility, bioaccumulation, and toxicity is highly dependent on the chemical species in which this element is present in the environment we consider that the proposed methodology opens new doors for future studies aimed at understanding the Hg biogeochemical cycle at the molecular scale. Future experiments applying the proposed methodology with conventional high resolution mass spectrometry might also provide interesting and complementary structural information about possible unknown Hg species to be analyzed in real samples.

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