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Approach to Measure Isotopic Ratios in Species Using Multicollector-ICPMS Coupled with Chromatography

Vladimir N. Epov,*,† Sylvain Berail,† Maria Jimenez-Moreno,†,‡ Vincent Perrot,† Christophe Pecheyran,† David Amouroux,† and Olivier F. X. Donard†

LCABIE, IPREM, Université de Pau et des Pays de l'Adour/CNRS UMR 5254, Hélioparc, 2 Avenue du Président Pierre Angot, Pau, 64053, France, and Department of Analytical Chemistry and Food Technology, University of Castilla-La Mancha, Faculty of Environmental Sciences, Toledo, Spain

A new approach was demonstrated for the isotope ratio measurement in different elemental species of Hg using transient signal obtained by chromatography coupled with multicollector-inductively coupled plasma mass spectrometry (MC-ICPMS). The method based on the slope of linear regression by transient intensities of different isotopes shows improved accuracy and reproducibility (0.2−0.5‰ as 2 standard deviation (SD)). Internal precision (RSD) of the method is very close to the theoretical value given by the counting statistic and is better by a factor of 6 in comparison with previous conventional methods of calculation. We demonstrated that internal RSD (uncertainty) depends on regression coefficients of the linear function (R^2). The typical internal precision of isotopic ratio measurements (0.003−0.02%) was achieved for δ^{202}Hg when injecting as low as 90 pg of Hg species. With the new methodology, it is possible to (i) measure the isotopic composition when a sample and a bracketing standard have significantly different concentrations, (ii) measure the isotopic composition of different species in samples versus single species in a bracketing standard, and (iii) measure the isotopic ratios for low abundant isotopes. We demonstrated application of this method for different environmental samples and processes.

Isotopic composition in different elemental species is a relatively new field in analytical chemistry. It can be used for interdisciplinary purposes in geo-sciences, biological sciences, ecology, environmental sciences, etc., since it can combine molecular and isotopic information tracking fractionation mechanisms. Recently, several new methods to measure species-specific isotopic ratios in some real-world samples using online chromatographic separation coupled with multicollector inductively coupled plasma mass spectrometry (MC-ICPMS) have been developed.[1−5] From one side, MC-ICPMS has become an advanced technique for the measurement of nontraditional isotopic compositions at high precision.[6,7] From another side due to the use of ICP as the ion source[8] MC-ICPMS instruments are perfectly suited for the hyphenation of chromatographic separation techniques. The last is particularly advantageous over other isotopic instruments such as thermal ionization mass spectrometry (TIMS).

The coupling of a chromatographic technique to the MC-ICPMS provides a transient signal within a specific time window in which the isotope ratios should be accurately measured. With dependence on the introduction technique applied, the duration and the intensity of the transient signal will be different and consequently this will influence the precision and accuracy of the isotope ratio measurement. Other sample introduction techniques that produce transient signal are gold-trap (GT),[9,10] laser ablation (LA),[11] or flow-injection (FI). Recently, Fietzke et al.[12] published a new acquisition and evaluation strategy which improves precision of Sr isotopic ratio measurement using LA-MC-ICPMS in carbonates. They have demonstrated that when collecting raw-data intensities for transient signal from the background to the maximum of the peak, the slope of the linear regression of the simultaneously measured intensities of two isotopes represents their isotopic ratio. This simple method[12] improves accuracy and precision and is advantageous versus conventional data reduction

* To whom correspondence should be addressed. E-mail: vladimir.epov@univ-pau.fr. Phone: +33 (0) 540 175 033. Fax: +33 (0) 559 407 781.

† Université de Pau et des Pays de l'Adour/CNRS UMR 5254.

‡ University of Castilla-La Mancha.

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protocols using separate integration of the peaks and/or data collection.

We can hypothesize that the method of calculation from Fietzke et al.[12] could be applied for chromatographic techniques coupled with MC-ICPMS due to the transient signal obtained during the chromatographic online separation. However, for the chromatography hyphenated with MC-ICPMS, there are several issues that should be investigated: (i) precision of the measurement due to the limited number of points (or peak width) obtained during the transient signal,[3] (ii) possible isotopic fractionation during the development of chromatographic peak due to the kinetic processes related with either chemical processes (reactions of species with chromatographic material) or physical processes (evaporation from the column surface),[13] (iii) conventional methods of the calculation of the isotopic ratio require the same concentration (±10%) of both the sample and bracketing standard, (iv) each species in the sample should be bracketed by the same species in the standard,[3] which requires one to obtain certified standards for each species.

The aim of this study was to investigate such a new methodology of calculation to be able to measure more precise isotopic ratios in species using chromatography coupled with MC-ICPMS and finding solutions for the issues described above. In this work, we used this new approach (i) to measure isotopic ratios when the species have significant differences in concentration and (ii) to measure δ-values in different species of the sample versus single species, presented in the standard. Also, we applied a new calculation strategy for Hg isotopic composition in four mercury species using gas chromatography (GC) coupled to MC-ICPMS.

Because of the toxicity of Hg, especially the organic form monomethylmercury, the study of Hg isotopes is nowadays an important way to understand the complex biogeochemical cycle of Hg. There are seven stable Hg isotopes with different natural abundances: ^{196}Hg (0.15), ^{198}Hg (9.97), ^{199}Hg (16.87), ^{200}Hg (23.10), ^{201}Hg (13.18), ^{202}Hg (29.86), and ^{204}Hg (6.87). Any variations in the isotopic composition of heavy elements, such as Hg, are to be small and difficult to measure. However recent appearance of modern mass spectrometry techniques, such as MC-ICPMS, allows the measurement of the six most abundant Hg isotopes[14] and simultaneous measurement of an independent Tl isotope pair to correct raw ratios for the mass bias. During the past decade, several studies demonstrated the evidence of both the mass-dependent and mass-independent natural fractionation of Hg isotopes in a variety of environmental materials and natural processes.[15] These studies have shown that variations in the Hg isotopic signatures may be used as tracers to distinguish between Hg sources and chemical transformations. Recommendations for reporting Hg isotope fractionations and uncertainties (as ^{xxx}Hg/^{198}Hg) were published by Blum and Bergquist[16] and are used in this manuscript. Furthermore, importance of Hg speciation in the environment has led to the development of techniques allowing one to study species-specific Hg isotopes signatures.[3,4]

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EXPERIMENTAL SECTION

Some experimental details of the measurement are described by Epov et al. in the previous paper.[3] The most important descriptions or modifications are presented below.

Reagents, Standards, and Samples. A standard reference material NIST-3133 was used to prepare the bracketing δ-zero standard. Secondary standards of inorganic mercury (F65A and RL24H)[17] were obtained from the CRPG (Nancy, France) for the validation of the Hg isotopic ratio measurements. Methylmercury prepared from the methylmercury chloride (Strem Chemicals) was used to validate the new method. A reference material of thallium NIST-997 was used to correct for the instrumental mass-bias. Biological reference materials IAEA-086 (human hair), IAEA-085 (human hair), and ERM-CE464 (tuna fish) have been used to measure isotopic composition using the new methodology.

Dimethylmercury was synthesized using the Grignard reaction with MeMgBr.[18] A stock solution of 100 mg L^{-1} $HgCl_2$ as Hg (>99%, p.a., Merck, Darmstand, Germany) was previously prepared directly in toluene from the purified compound. An aliquot of 1 mL of this solution was cooled on an ice/water bath. Then the Grignard reagent (0.4 mL of a 3.0 mol L^{-1} methylmagnesium bromide solution in diethyl ether (Aldrich Chemie, Steinheim, Germany)) was added. The tube remained in the ice/water bath for 5 min with occasional shaking. Subsequently, the reaction was quenched by the rapid addition of 0.4 mL of 0.6 mol L^{-1} hydrochloric acid.

For the GC-MC-ICPMS analysis, propylation was chosen for the derivatization of the Hg species.[3] For the total isotopic analysis of Hg, all species of Hg were transformed to inorganic mercury (IHg^{2+}) using digestion with HNO_3 and followed with the addition of BrCl. To neutralize excess of BrCl, a 30% solution of hydroxylamine was added before the introduction of the sample to the cold-vapor generation system coupled to MC-ICPMS. To convert IHg^{2+} to elemental mercury (Hg^0) using cold-vapor-generation (CVG), a 3% solution of $SnCl_2 \cdot 2H_2O$ in 1 N HCl was used.

Instrumentation. A Nu Plasma HR MC-ICPMS from Nu Instruments (Wrexham, U.K.) was employed to measure isotopic ratios of Hg. Operating parameters used for this instrument are presented in Table 1. The detector configuration presented in this table allows one to simultaneously measure seven stable Hg isotopes, two Tl isotopes to correct for a mass-bias and two Pb isotopes to correct for interference at $m/z = 204$. The instrument was equipped with a commercially produced double-inlet torch which allows the coupling of a GC system Focus from Thermo Fisher Scientific (Milan, Italy) and the simultaneous introduction of a Tl solution with a 200 μL min^{-1} self-aspirating microconcentric nebulizer coupled to a cinnabar spray chamber. GC parameters are also presented in Table 1. For the acquisition of the transient signals, we used the time resolved analysis mode of the Nu Plasma HR with an integration time of 0.5 s.

For the total isotopic analysis of Hg, a HGX-200 cold vapor system and an ASX-110FR autosampler both from CETAC (Omaha, NE) was used to introduce mercury. A DSN-100 desolvation nebulizer system from Nu Instruments (Wrexham,

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Table 1. Instrumental Parameters

Nu Plasma HR	
plasma gas flow rate	13 L min^{-1}
auxiliary gas flow rate	0.9 L min^{-1}
nebulizer gas pressure (psi)	16.4
rf power	1300 W
interface cones	nickel WET plasma cone (type A)
acceleration voltage	6 kV
instrument resolution	low resolution
high voltage and source ion lenses	optimized for maximum intensity and stability of the signal
quad lenses	optimized for the best flat top peak shape and best coincidence

Faraday Cups Configuration

H7	H6	H5	H4	H3	H2	H1	Ax	L1	L2	IC0	L3	IC1	IC2	L4
208	206	205	204	203	202	201	200	199	198	–	196	–	–	–

GC Focus	
column	MXT, 30 m, 0.53 mm i.d., 1.0 μm coating
injector type	split/splitless
injector volume	2 μL (mode splitless)
injector temperature	250 °C
He carrier gas flow rate	25 mL min^{-1}
additional Ar make up gas flow rate	0.4 L min^{-1}
GC temperature program	
initial temperature	40 °C
initial time	7 min
ramp 1	60 °C min^{-1}
final temperature 1	80 °C
hold time 1	6 min
ramp 2	60 °C min^{-1}
final temperature 2	250 °C
hold time 2	1 min

U.K.) and a double-inlet torch were used to introduce Hg and Tl simultaneously.

Calculation. In this work we used the "standard−sample−standard" bracketing technique to calculate δ-values for Hg isotopes[16] and Russell correlation to correct for the mass-bias using Tl isotopes.[3] δ values (δ^{204}Hg, δ^{202}Hg, δ^{201}Hg, δ^{200}Hg, δ^{199}Hg, and δ^{196}Hg) were calculated for the isotopic ratios ^{204}Hg/^{198}Hg, ^{202}Hg/^{198}Hg, ^{201}Hg/^{198}Hg, ^{200}Hg/^{198}Hg, ^{199}Hg/^{198}Hg, and ^{196}Hg/^{198}Hg, respectively. Also, Δ^{xxx}Hg values were calculated to verify mass-independent fractionation.[16] In addition, interference from ^{204}Pb at $m/z = 204$ was corrected using natural abundances of ^{208}Pb, ^{206}Pb, and ^{204}Pb isotopes and total intensities (^{208}I, ^{206}I, and ^{204}I) at m/z values = 208, 206, and 204, respectively,

$$I(^{204}\mathrm{Hg}) = {}^{204}I - \frac{{}^{208}I \times 0.0267 + {}^{206}I \times 0.0581}{2} \qquad (1)$$

Three methods of calculation of the isotopic ratios were compared for precision, accuracy, and flexibility of the analytical methodology: (1) peak area integration (PAI), using the area of the chromatographic peak as it was described in previous work;[3] (2) point by point (PbP), calculation of average isotopic ratios for several points of selected peak area; (3) linear regression slope (LRS), new method of calculation, using slope of the line obtained from signal intensities of two isotopes (Figure 1); details of the method are described in the Results and Discussion. The three methods of calculation were applied for the different zones of the peak, similar to those described by Epov et al.[3] and also presented in Figure 2. For example, to find 50% of the peak zone, we took into account all the intensities higher than 50% of the peak maximum. For all three methods of calculation, the background was taken into account by the subtraction of the baseline before the elution of the peak. The raw data ratios were corrected for the instrumental mass-bias and then used for the calculation of isotopic composition (δ values) of different species.

RESULTS AND DISCUSSION

Method of Calculation. The new approach of isotopic ratios calculation in species (method 3 from the Experimental Section) was based on the method recently published by Fietzke et al.,[12] where authors describe precise measurement of the Sr isotope ratio using LA-MC-ICPMS. Here, we developed a calculation for the isotope ratio analysis of different Hg species after online separation using chromatography. An example of the calculation of Hg isotopic ratios (^{204}Hg/^{198}Hg, ^{202}Hg/^{198}Hg, ^{201}Hg/^{198}Hg, ^{200}Hg/^{198}Hg, ^{199}Hg/^{198}Hg, and ^{196}Hg/^{198}Hg) for NIST-3133 standard is demonstrated in Figure 1. The axis of abscissa represents the signal intensity (in volts) for the ^{198}Hg isotope, which is the denominator of isotopic ratios as recommended by Blum and Bergquist.[16] The axis of ordinate represents signal intensities for other Hg isotopes (^{204}Hg, ^{202}Hg, ^{201}Hg, ^{200}Hg, ^{199}Hg, and ^{196}Hg), which are the nominators of isotopic ratios. The isotopic ratio of Hg isotopes are calculated similar to the calculation described by Fietzke et al.[12] using the INDEX(LINEST(.. .)) function of MS EXCEL. The intensity weighted contribution between the on-peak-zero baseline and the maximum intensity of the peak is also described in the latter paper.

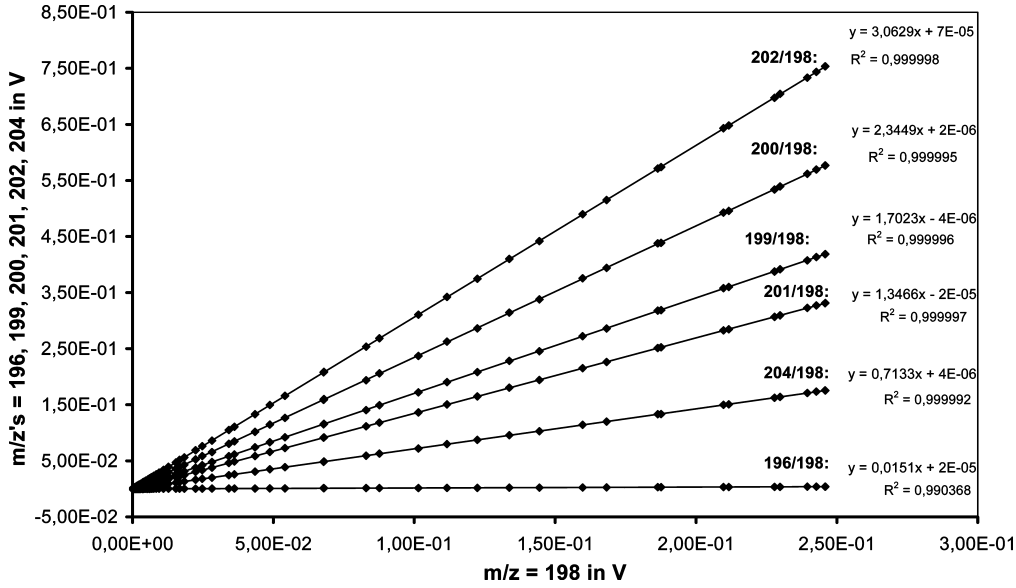


Figure 1. Intensities at $m/z = 196$, 199, 200, 201, 202, and 204 versus intensity at $m/z = 198$ for the chromatographic peak obtained for 300 pg of IHg^{2+} measured by GC-MC-ICPMS. Slope of each equation represents isotopic ratios for Hg isotopes ($^{196}Hg/^{198}Hg$, $^{199}Hg/^{198}Hg$, $^{200}Hg/^{198}Hg$, $^{201}Hg/^{198}Hg$, $^{202}Hg/^{198}Hg$, and $^{204}Hg/^{198}Hg$).

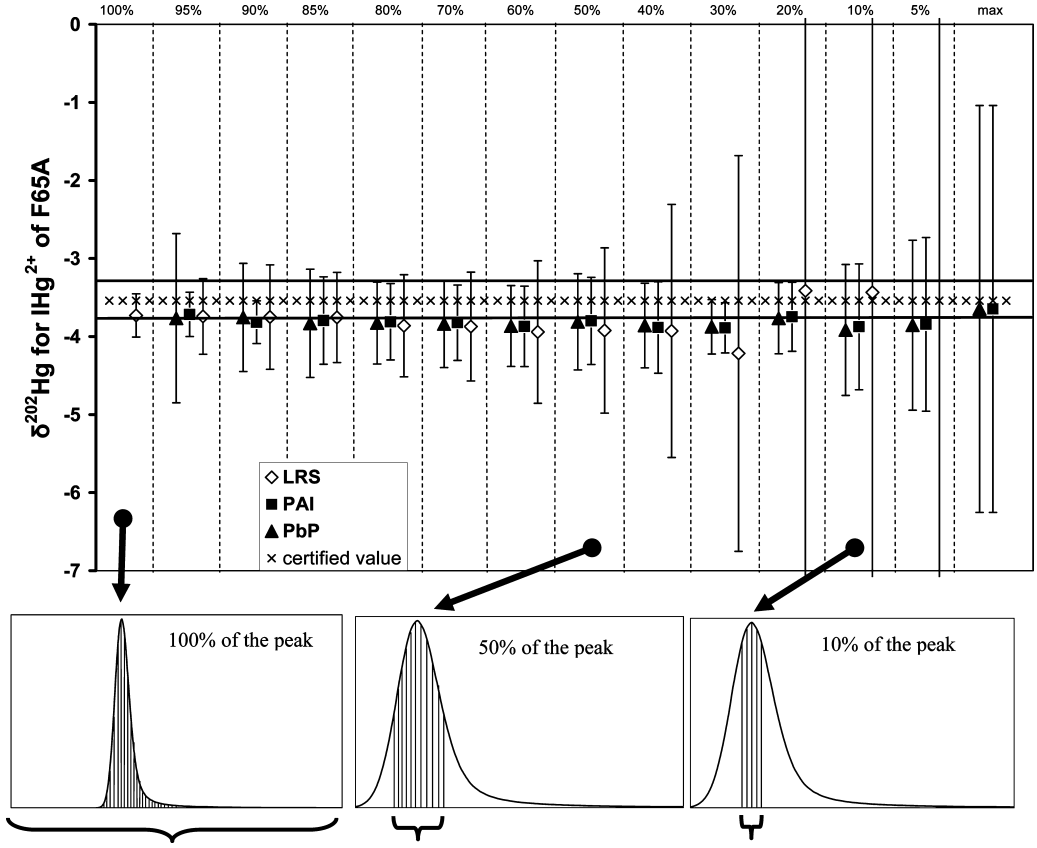


Figure 2. $\delta^{202}Hg$ (in per thousand (‰)) values calculated for IHg^{2+} species of F65A secondary standard using three different methods for different peak zones: (i) using each point of the peak (PbP); (ii) using integration of the peak (PAI); (iii) using slope created by intensities of the isotopes (LRS). Uncertainties represent 2SD (‰) of 33 replicates. Examples of the selection of peak zones are presented at the bottom of the figure for 100, 50, and 10% of the peak and described in the text.

Different isotopic ratios of Hg measured using the LRS method of calculation are presented in Table 2. The linear regression slope line is described by eq 2:

$$y = (b \pm u_b)x + (a \pm u_a) \quad (2)$$

where x and y are the denominator and nominator in isotopic ratios, respectively; b is the slope of the linear regression line representing the isotopic ratio; a is the intercept of the line; u_b and u_a are uncertainties of the measurements of b and a, respectively. All the parameters were calculated by using the

Table 2. Isotopic Ratios and Other Analytical Parameters Calculated for the Single Injection of 400 pg of IHg^{2+} Species As the Slope of $y = (b \pm u_b)x + (a \pm u_a)$[a]

parameter	b (value of isotopic ratio)	u_b	a	u_a	R^2
$^{204}Hg/^{198}Hg$	0.699 48	0.000 15	0.000 016	0.000 005	0.999 986
$^{202}Hg/^{198}Hg$	3.021 9	0.000 4	0.000 078	0.000 013	0.999 994
$^{201}Hg/^{198}Hg$	1.332 3	0.000 2	0.000 017	0.000 006	0.999 993
$^{200}Hg/^{198}Hg$	2.328 8	0.000 4	0.000 033	0.000 011	0.999 993
$^{199}Hg/^{198}Hg$	1.696 2	0.000 3	0.000 083	0.000 008	0.999 993
$^{196}Hg/^{198}Hg$	0.015 04	0.000 13	−0.000 009	0.000 004	0.977 558
$^{200}Hg/^{202}Hg$	0.770 62	0.000 06	−0.000 026	0.000 006	0.999 998
	Calculated for $^{202}Hg/^{198}Hg$ Using Different Peak Zones				
100%	3.021 9	0.000 4	0.000 078	0.000 013	0.999 994
95%	3.022 6	0.000 8	0.000 011	0.000 065	0.999 997
90%	3.023 4	0.001 1	−0.000 069	0.000 098	0.999 996
85%	3.022 2	0.001 2	−0.000 063	0.000 12	0.999 995
80%	3.022 6	0.001 5	−0.000 021	0.000 16	0.999 994
70%	3.022 6	0.002 2	0.000 023	0.000 25	0.999 990
60%	3.022 9	0.001 4	−0.000 032	0.000 16	0.999 996
50%	3.022 5	0.003 8	0.000 037	0.000 48	0.999 978
40%	3.019 5	0.005 3	0.000 46	0.000 72	0.999 965
30%	3.018 8	0.007 1	0.000 56	0.000 98	0.999 950
20%	3.022 7	0.010 8	−0.000 017	0.001 54	0.999 911
10%	3.033 0	0.010 9	−0.001 54	0.001 62	0.999 948

[a] Where b is the slope of the linear regression (isotopic ratio), a is the intercept, and u_b and u_a are the uncertainties of b and a, respectively.

"least squares" method of regression analysis in Excel. The u_b uncertainty represents internal precision of the single measurement of isotopic ratios. As can be seen from Table 2, the value of this uncertainty is related with the abundance of isotopes. For example, when the higher abundance isotope is used as the denominator ($^{200}Hg/^{202}Hg$), better internal precision is obtained. However in this paper, we used ^{198}Hg as the denominator as it was recommended.[16] Also, it can be seen from this Table that R^2 is lower for lower abundant isotopes.

Figure 2 demonstrates the $\delta^{202}Hg$ accuracy and external precision of the developed LRS method in comparison with conventional methods of calculation, such as PAI (peak area integration) and PbP (average isotopic ratios for several points of the peak). The secondary standard (F65A) and the bracketing standard (NIST-3133) were measured with the same intensity, equal to approximately 2 V for the ^{202}Hg in 33 replicates within 2 weeks. Different peak-zones were chosen for the calculation of isotopic ratios for each GC injection. Then the instrumental mass-bias was corrected using the average $^{205}Tl/^{203}Tl$ signal for the same peak zone. Finally δ values were calculated for F65A relative to NIST-3133 with 33 replicates during 3 weeks. A mass-dependent isotopic composition was observed for the F65A, and results are in a good agreement with those previously published by Estrade et al.[17] Accuracy of the measurement is identical for all the calculations, but precision (2SD) of the measurement was significantly different. The best precision was observed for the peak zone of 100% using the LRS method of calculation and for the peak zone of 95% or 90% using the PAI method. For example, for the peak zone of 100% using LRS, the uncertainty was equal to 0.28‰ as 2SD for $\delta^{202}Hg$, which is comparable with uncertainty observed by the continuous signal MC-ICPMS measurement (0.1−0.3‰). Higher uncertainty was found for the PbP method or for other peak zones of LRS or PAI methods. When the peak zone is shorter, uncertainty for all three methods is increasing. For smaller peak zones (5−80% of the peak), the LRS method has worst precision among three methods of calculation. This demonstrates the importance of using on-peak-zero-baseline (background) for the LRS method of calculation. Also, we have compared internal uncertainty and R^2 parameters when calculating different peak zones using the LRS method. As can be seen from the Table 2 with the decreasing of the peak-zone, uncertainty is increasing and the R^2 value is decreasing.

To conclude this part of the manuscript, we can state that PAI and LRS methods give similar results in terms of external precision and accuracy when measuring a similar concentration of the same species in the sample and in the standard. The main advantage of the LRS method is that it does not require determining the integration limits of the peak. The PbP method is limited by the number of points which can be used for the average isotopic ratios within the peak, since it is limited by the width of the transient signal. However, increasing the number of points by widening the chromatographic peak leads to a decrease of the signal intensity and sometimes peak resolution.

Measurement of Isotopic Composition for Different Concentration Ratio of Sample/Standard. When a sample and a bracketing standard have different concentration during the measurement, the LRS method demonstrates significant advantage in terms of accuracy and precision. Figure 3 demonstrates the veracity and external reproducibility of $\delta^{202}Hg$ for three different methods of calculation when the ratio of sample/standard concentrations is significantly different from unity. Accuracy (Y-axis) was calculated as the difference of a calculated δ value from the δ value published by Estrade et al.[17] in per thousand (‰). Reproducibility (error bars) was calculated as 2SD values (‰) for three replicates.

If the signal of the sample is 4 times higher than the signal of the standard, δ-values for both PAI and PbP methods (95% of the peak were used for both methods) are significantly inaccurate. The LRS method gives accurate value with good precision.

When the signal of the standard is 5 times higher than the signal of the sample, the uncertainty of the PAI and PbP methods was found to be very high. For the LRS method of calculation, both reproducibility (0.18‰ as 2SD) and accuracy was found to

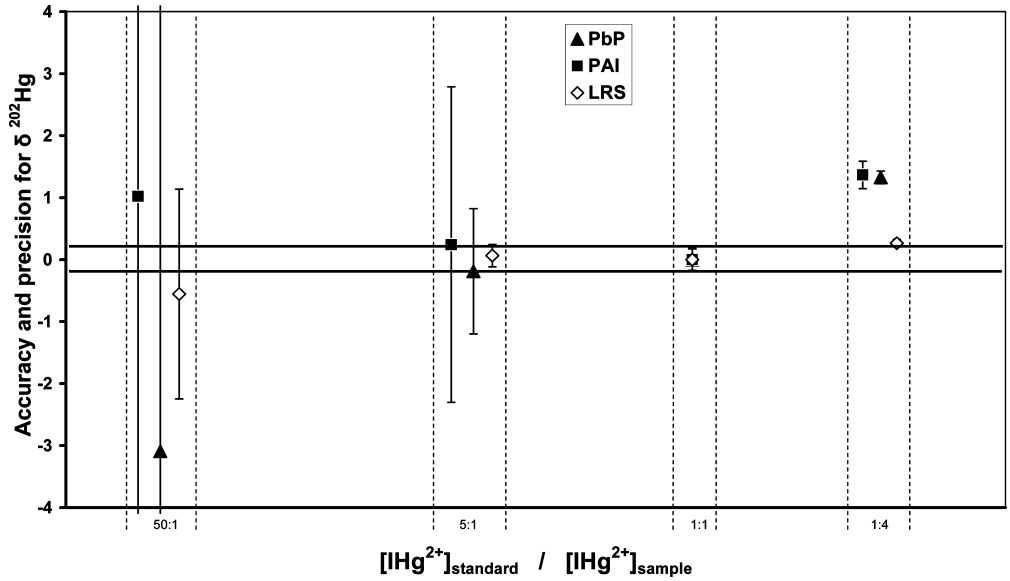


Figure 3. Accuracy and precision of δ values measured using three different methods of calculation for the F65A relative to the NIST-3133 standard when having different concentration ratios of $[IHg^{2+}]_{NIST\text{-}3133}/[IHg^{2+}]_{F65A}$. Uncertainties represent 2SD (‰) of three replicates.

be very good. If the concentration of standard was 50 times higher than the concentration of the sample, uncertainty of the δ values was found to be very high for all three methods of calculation. However, for this last case, precision and accuracy of the LRS method (1.7‰ as 2SD) was still significantly better than for the calculation using PAI and PbP methods (17‰ and 21‰ as 2SD, respectively).

Better results for the LRS method could be explained by the counting statistic, since internal precision of the results is critically dependent on it. External precision (or reproducibility) of the method will be better if internal precision of the method is lower. For isotopic ratios, theoretical internal uncertainty can be calculated using eq 3, where I_1 and I_2 are the number of counts for isotopes 1 and 2, respectively:

$$\mathrm{RSD}(\%) = 100 \times \sqrt{\frac{1}{I_1} + \frac{1}{I_2}} \qquad (3)$$

Internal precision calculated as a relative standard deviation (RSD, %) of the $^{202}Hg/^{198}Hg$ isotopic ratio for different amounts of Hg injected into the MC-ICPMS is presented in Figure 4a. The theoretical RSD was calculated using eq 3 for the amount of Hg analyzed. The internal uncertainty of the PbP method was calculated as RSD (%) of isotopic ratios for several points within the peak. The internal uncertainty of the continuous signal for the same amount of Hg measured by CVG-MC-ICPMS was calculated as RSD (%) of the isotopic ratios for 30 cycles during 10 s. The internal uncertainty of the LRS method was calculated using the SD of the slope value given by the LINEST function of MS EXCEL. Figure 4a demonstrates that internal precision of the LRS method of calculation is significantly better (6 times) than the one of the PbP method and much closer to the theoretical internal precision. This is due to the fact that for the PbP method all the ratios have the same weight even if the signal intensity at the peak edges is poor leading to the higher uncertainty. Whereas, the LRS method provides a natural weighting of the points, favoring the most intense signal zone compared to the peak edges and hence minimizing internal precision. Moreover, internal uncertainty for the LRS method is lower (3−4 times) than the internal uncertainty observed for continuous CVG-MC-ICPMS measuring same amount of Hg and in the same range with the typical internal uncertainty (0.003−0.02%) of continuous MC-ICPMS isotopic ratio measurements.

As it was demonstrated in Table 2, internal uncertainty of isotopic ratios obtained by the LRS method has the relationship with the R^2 parameter. Figure 4b shows this relationship of internal precision (presented as RSD) versus the regression coefficient (R^2) of the linear function created by the intensities of two isotopes. A higher value of R^2 gives lower internal RSD, having a polynomial trend of the second order. Typical internal RSD of continuous MC-ICPMS measurement (0.003−0.02%) can be achieved when R^2 is equal to 0.999 983−0.999 999, which corresponds to injection of 90−3200 pg of Hg species for the $^{202}Hg/^{198}Hg$ ratio.

To conclude this part of the paper, we can state that one of the novelties of the LRS method is the possibility to apply it when the bracketing standard and the sample have a significantly different concentration keeping acceptable reproducibility and accuracy. Moreover, in these cases precision and accuracy observed for the LRS method are better than continuous signal isotopic measurements for the same amount of Hg.

Measurement of Isotopic Composition between Different Species versus Single Species. We studied the possibility to measure the isotopic composition in different species from the chromatogram of the sample (Figure 5) relative to the only species of the bracketing standard having certified isotopic values. The LRS method of calculation is suitable for this bracketing, since 100% of the chromatographic peak (all the data from the background to the maximum of the peak) is included and hence full isotopic ratio of all injected species is taken into account (Figure 5). For the PbP and sometimes for PAI methods, accuracy and precision could be worsened by removing/varying the peak edges during the calculation.

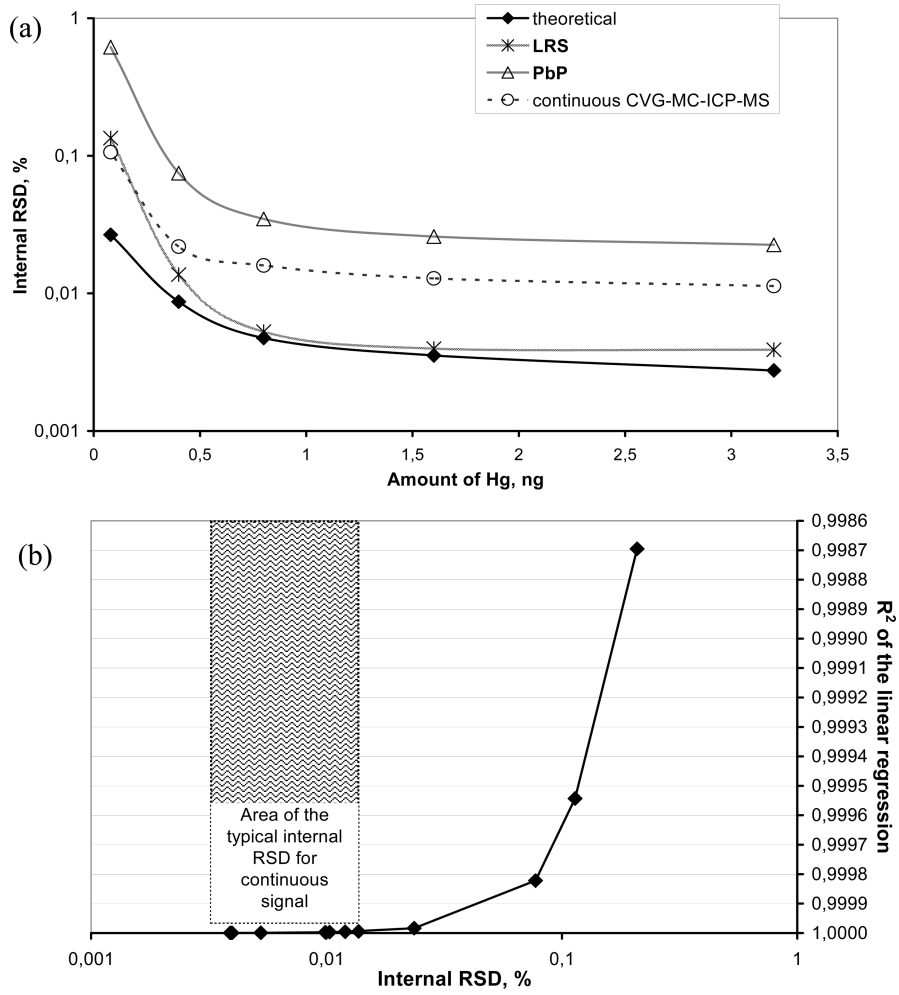


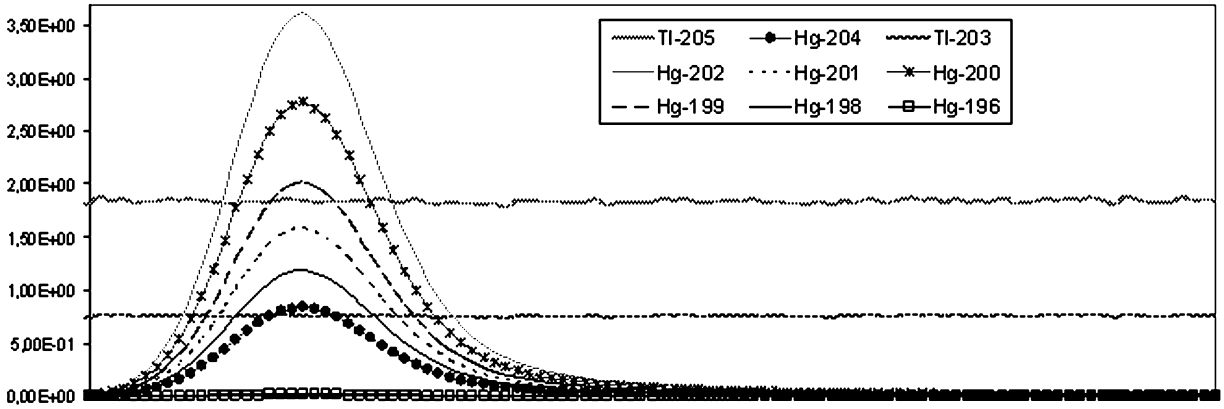
Figure 4. (a) Comparison of internal precision for PbP and LRS methods using GC-MC-ICPMS measurement, for continuous signal using CVG-MC-ICPMS measurement, and for theoretically calculated values; the precision is presented as a relative standard deviation (RSD, %) for different amount of Hg analyzed. (b) Internal RSD of the LRS method versus regression coefficient (R^2) of the linear function created by the intensities of two isotopes; selected area is the typical RSD for the isotopic ratio measurements using continuous signal CVG-MC-ICPMS.

In Table 3, we compare the results of the isotopic composition calculation of the methylmercury (MeHg) species (STREM) relative to IHg^{2+} (NIST-3133) using three different methods. These results were also compared with the values obtained by the CVG coupled with MC-ICPMS and with previously published δ-values for the STREM standard.[3] The LRS method of calculation gives the best accuracy and precision (-0.68 ± 0.33‰ for δ^{202}Hg) of the results in comparison with two other methods of calculation (δ^{202}Hg, -0.73 ± 0.94‰ and -0.78 ± 0.49‰ for PbP and PAI, respectively). All δ values for the LRS method show mass-dependent isotopic composition with the precision in the range of 0.25−0.56‰ (calculated as 2SD) depending on the abundance of Hg isotope. Values of Δ^{xxx}Hg presented in this table also demonstrate the advantage of the LRS method over two other methods, since this method gives more accurate and precise results. In addition, the results are in a good agreement with CVG-MC-ICPMS measurements presented both in Table 3 (δ^{202}Hg, -0.63 ± 0.15‰) and published by Epov et al. (δ^{202}Hg, -0.50 ± 0.29‰).[3]

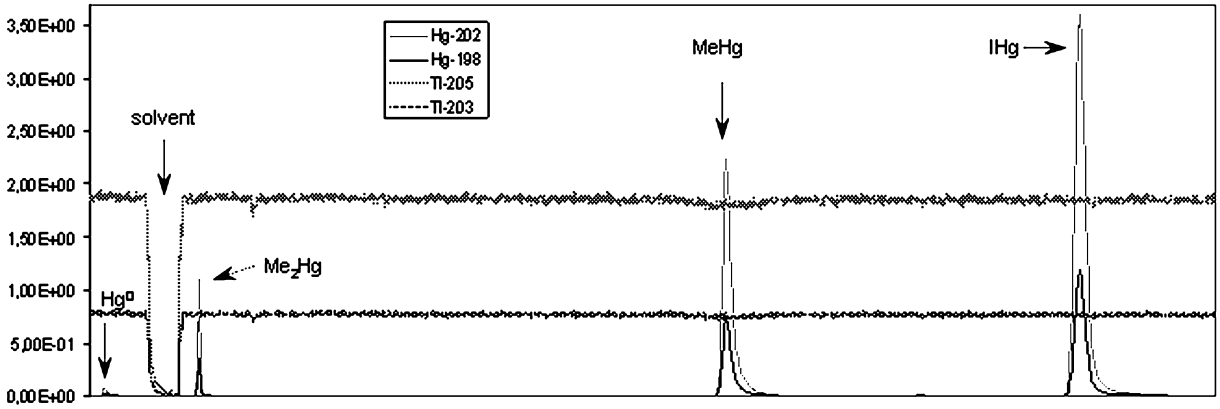
The main conclusion of this part of the manuscript is that the LRS method of calculation can be used to measure δ values between different species with good precision and accuracy. The results are advantageous in comparison with the PbP and PAI methods. Also it could be advantageous in comparison with continuous signal isotopic measurement, since the LRS method is the online multiple-species precise isotopic analysis which is much simpler and much more robust due to avoiding offline separation of the species.

Possibility to Measure Low Abundance Isotopes. Another advantage of the LRS method of calculation is the possibility to measure isotopic composition for the low-abundance isotopes, even when the abundance of this isotope and hence total height of the peak are low. This fact has been discussed by Fietzke et al. for the low abundance ^{84}Sr isotope using LA-MC-ICPMS.[12] Here, we demonstrated for GC-MC-ICPMS that δ^{204}Hg can be measured precisely (0.2−0.6‰ as 2SD) and accurately, showing the mass-dependent isotopic composition for the measured samples (Figure 1 and Tables 3 and 4). Also, it is possible to sometimes measure the isotopic composition of δ^{196}Hg (natural abundance equal to 0.153%) when the amount of Hg species injected to GC-MC-ICPMS is higher than 800 pg (Figure 1). For example, in Table 4 results for δ^{196}Hg in F65A and RL24H relative to the NIST-3133 standard are presented. Mass-dependent values for δ^{196}Hg were observed (see Δ values) with satisfactory accuracy and precision equal to 0.6−0.8‰ as 2SD.

1. Standard NIST-3133 (single IHg^{2+} species) injected to GC and NIST-997 (Tl) introduced to spray-chamber for MC-ICP-MS measurement:



2. Sample (multi-species with different concentrations) injected to GC and NIST-997 (Tl) introduced to spray-chamber for MC-ICP-MS measurement:



3. Polyatomic and/or isobaric interferences corrected using equation (1)

4. Isotopic ratios calculated using slope of linear regression created by intensities of the transient signal of 2 isotopes between on-peak-zero baseline and the maximum intensity of the peak: see Figure 1

5. Mass-bias corrected using Tl and Russell correlation

6. Isotopic composition (δ-values) calculated for each species using bracketing technique

Figure 5. Schematic diagram of the isotopic composition measurement using the LRS method.

However, the possibility to measure the isotopic composition of low abundance isotopes will depend on the signal level.

As it was discussed above, internal precision of the LRS method is related to the regression coefficient (R^2) of the linear function created by the intensities of two isotopes (Figure 4b). For the amount of Hg species equal to 300 pg (Figure 1), regression coefficients (0.999 99) are similar for five linear functions created by the intensities of ^{202}Hg, ^{200}Hg, ^{199}Hg, ^{201}Hg, and ^{204}Hg versus intensities of ^{198}Hg, though the abundance of ^{204}Hg is 4.3 times lower than the abundance of ^{202}Hg. Only for the linear function

Table 3. Mercury Isotopic Composition (δ Values) of MeHg STREM Standard Relative to IHg^{2+} NIST-3133 Measured by GC-MC-ICPMS and Calculated Using Different Methods and Compared with Conventional CVG-MC-ICPMS Measurement[a]

method	δ^{202}Hg	δ^{204}Hg	Δ^{204}Hg	δ^{201}Hg	Δ^{201}Hg	δ^{200}Hg	Δ^{200}Hg	δ^{199}Hg	Δ^{199}Hg
CVG-MC-ICPMS	−0.63 ± 0.15	−0.92 ± 0.29	0.02 ± 0.26	−0.49 ± 0.12	0.02 ± 0.10	−0.30 ± 0.08	0.01 ± 0.06	−0.14 ± 0.04	0.02 ± 0.04
				GC-MC-ICPMS					
PbP	−0.73 ± 0.94	−1.86 ± 12.0	−0.76 ± 11.8	−0.49 ± 1.05	0.07 ± 0.72	−0.31 ± 1.03	0.06 ± 0.78	−0.13 ± 0.94	0.06 ± 0.80
PAI	−0.78 ± 0.49	−1.44 ± 2.23	−0.28 ± 2.11	−0.48 ± 1.14	0.11 ± 1.08	−0.53 ± 2.03	−0.14 ± 2.00	−0.32 ± 2.06	−0.13 ± 2.04
LRS	−0.68 ± 0.33	−1.06 ± 0.56	−0.02 ± 0.56	−0.48 ± 0.35	0.03 ± 0.27	−0.33 ± 0.25	0.01 ± 0.14	−0.14 ± 0.27	0.03 ± 0.24

[a] Uncertainties represent 2SD (‰) for 52 replicates.

created by the intensities of the ^{196}Hg and ^{198}Hg isotopes, the regression coefficient is lower (0.990 36) due to the significantly lower abundance of the ^{196}Hg isotope (200 times less abundant than ^{202}Hg). Hence the internal precision of the ^{196}Hg/^{198}Hg measurement was significantly lower. From Figure 4b, we can conclude that to achieve good internal uncertainty (<0.08%) for the ^{196}Hg/^{198}Hg ratio, the amount of injected Hg should be higher than 3 ng, which will correspond to the linear regression coefficient equal to 0.9998.

In this part of the paper we can conclude that it is possible to measure low abundance isotopes in different species when applying hyphenation of a chromatographic technique with MC-ICPMS and the LRS method of calculation. The results are advantageous relative to other methods of calculation and relative to the conventional method of continuous sample introduction, which often gives higher internal uncertainty when measuring low abundance isotopes. This advantage of the LRS method is related with the fact that internal precision of this method depends on the R^2 of the slope.

Applications of the LRS Method to Synthetic and Natural Samples. In this section we demonstrate applications of the LRS method to measure the isotopic composition of Hg in different species of different environmental and biological samples. A detailed schematic description of this measurement is presented in Figure 5: (i) bracketing standard, such as NIST-3133, representing a single species (IHg^{2+}) is introduced before and after each sample; (ii) sample represents several species with different concentrations, such as Hg^0, Me_2Hg, MeHg, and IHg^{2+}; (iii) interferences are corrected both for the standard and for the sample using eq 1; (iv) isotopic ratios in species (^{204}Hg/^{198}Hg, ^{202}Hg/^{198}Hg, ^{201}Hg/^{198}Hg, ^{200}Hg/^{198}Hg, ^{199}Hg/^{198}Hg, and ^{196}Hg/^{198}Hg) are calculated by the slope of the linear function created by the intensities of the pair of isotopes; (v) mass-bias is corrected using the Tl isotopic ratios measured simultaneously with the Hg species applying Russell correlation or exponential law; (vi) δ- and Δ-values are calculated for each species relative to NIST-3133. Results of the measurement for different samples are presented in Table 4.

Isotopic Composition of Hg(0) and Hg(II) in Secondary Standards. As a first application, we have measured the isotopic composition in old F65A and RL24H secondary standards, which are initially solely composed by inorganic IHg^{2+} species. After injection to GC, a low signal of elemental Hg^0 for F65A and a higher signal of Hg^0 for RL24H was measured in these secondary standards. We can assume that Hg^0 species were produced either due to the degradation of these two standards during the storage or during the derivatization procedure which is dependent on the experimental conditions.[19] If the first assumption is true, some Hg^0 also can be lost from the vials due to the volatilization. The isotopic compositions of both IHg^{2+} and Hg^0 species relative to NIST-3133 have been measured. The results presented in Table 4 demonstrate that for IHg^{2+} species we were able to measure the isotopic composition for all seven stable Hg isotopes, including the low abundance ^{196}Hg.

Isotopic composition of the IHg^{2+} species in F65A was found to be δ^{202}Hg = −3.73 ± 0.28‰ with mass-dependency for all Hg isotopes. This composition is statistically similar to those published by Estrade et al.[17] δ^{202}Hg = −3.54 ± 0.27‰ with mass-dependency for all Hg isotopes. Also, these values are statistically similar to those measured in this work using CVG-MC-ICPMS δ^{202}Hg = −3.53 ± 0.33‰ (n = 3) with mass-dependency for all Hg isotopes.

Isotopic composition of IHg^{2+} in RL24H was found to be δ^{202}Hg = 3.06 ± 0.54‰ with mass-dependency for all Hg isotopes. This composition is slightly more positive than those previously published[17] δ^{202}Hg = 2.59 ± 0.19‰ and those measured in this work using CVG-MC-ICPMS for the fresh RL24H standard δ^{202}Hg = 2.79 ± 0.22‰ (n = 3) with mass-dependency for all Hg isotopes.

Also using the LRS method, we were able to measure the isotopic composition of the Hg^0 species for the six most abundant Hg isotopes (Table 4). Results demonstrate the mass-dependent fractionation for all Hg isotopes and Hg^0 species. However, the isotopic composition of Hg^0 was found to be more negative than IHg^{2+} for both F65A (δ^{202}Hg = −5.41 ± 0.35‰) and RL24H (δ^{202}Hg = 2.36 ± 0.59‰). This can be explained by the fact that during degradation of IHg^{2+} to Hg^0 in dark conditions (during storage or sample preparation), negative mass dependent fractionation takes place in the vials. In other words, during the reduction of IHg^{2+} to Hg^0, light isotopes react faster, hence the reagent (IHg^{2+}) becomes enriched in heavier isotopes and the product (Hg^0) becomes enriched in lighter isotopes. The last is in agreement with previously published results[15,17] and with classical mass-dependent isotopic fractionation theory.

To check the accuracy, we have measured the total isotopic composition of old secondary standard RL24H by CVG-MC-ICPMS. δ^{202}Hg was found to be 2.97 ± 0.26‰ with mass-dependency for all Hg isotopes), which is in a good agreement with the results obtained by GC-MC-ICPMS (Table 4).

(19) Martin-Doimeadios, R. C. R.; Krupp, E.; Amouroux, D.; Donard, O. F. X. *Anal. Chem.* **2002**, *74* (11), 2505–2512.

Table 4. Application of the LRS Method: δ Values Measured and for Different Hg Species Relative to the NIST-3133 Standard[a]

species	δ^{202}Hg	δ^{204}Hg	Δ^{204}Hg	δ^{201}Hg	Δ^{201}Hg	δ^{200}Hg	Δ^{200}Hg	δ^{199}Hg	Δ^{199}Hg	δ^{196}Hg	Δ^{196}Hg
					F65A						
IHg^{2+}	−3.73 ± 0.28	−5.54 ± 0.52	0.02 ± 0.41	−2.80 ± 0.36	0.00 ± 0.26	−1.84 ± 0.27	0.04 ± 0.16	−0.88 ± 0.29	0.06 ± 0.23	2.27 ± 0.82	0.40 ± 0.75
Hg^0, degraded	−5.41 ± 0.35	−8.12 ± 1.63	−0.03 ± 1.37	−4.00 ± 0.70	0.08 ± 0.54	−2.76 ± 1.14	−0.04 ± 1.19	−1.29 ± 1.41	0.08 ± 1.44		
					RL24H						
IHg^{2+}	3.06 ± 0.54	4.64 ± 0.88	0.07 ± 0.44	2.14 ± 0.41	−0.16 ± 0.33	1.51 ± 0.31	−0.02 ± 0.23	0.61 ± 0.30	−0.16 ± 0.30	−1.41 ± 0.95	0.13 ± 2.04
Hg^0, degraded	2.36 ± 0.59	3.62 ± 1.67	0.09 ± 1.98	2.05 ± 0.63	0.27 ± 0.80	1.28 ± 2.45	0.09 ± 1.89	0.76 ± 1.16	0.16 ± 1.03		
					Synthesized $(Me)_2Hg$						
$(Me)_2Hg$	−0.80 ± 0.26	−1.23 ± 0.29	−0.04 ± 0.12	−0.45 ± 0.10	0.15 ± 0.10	−0.27 ± 0.28	0.13 ± 0.17	−0.04 ± 0.36	0.16 ± 0.30		
					IAEA-085 (Human Hair)						
MeHg, 98.7%	−0.48 ± 0.24	−0.71 ± 0.28	0.01 ± 0.14	−0.42 ± 0.14	−0.06 ± 0.07	−0.27 ± 0.24	−0.03 ± 0.12	−0.13 ± 0.12	−0.01 ± 0.06		
IHg^{2+}, 1.3%	−0.31 ± 0.12	−0.33 ± 0.16	0.13 ± 0.10	−0.24 ± 0.08	−0.01 ± 0.06	−0.25 ± 0.11	−0.09 ± 0.07	−0.04 ± 0.06	0.04 ± 0.03		
					IAEA-086 (Human Hair)						
MeHg, 45%	1.70 ± 0.24	2.53 ± 0.23	−0.01 ± 0.41	1.77 ± 0.25	0.49 ± 0.09	0.85 ± 0.37	−0.00 ± 0.26	1.09 ± 0.07	0.67 ± 0.01		
IHg^{2+}, 55%	0.25 ± 0.23	0.42 ± 0.27	0.08 ± 0.08	0.04 ± 0.06	−0.15 ± 0.11	0.04 ± 0.10	−0.09 ± 0.01	−0.10 ± 0.06	−0.17 ± 0.11		
					ERM-CE464 (Tuna Fish)						
MeHg, 97.65%	0.55 ± 0.24	0.93 ± 0.61	0.10 ± 0.26	2.38 ± 0.19	1.97 ± 0.26	0.37 ± 0.04	0.10 ± 0.08	2.52 ± 0.09	2.41 ± 0.15		
IHg^{2+}, 2.35%	−2.18 ± 0.38	−3.21 ± 0.51	0.04 ± 0.37	−0.36 ± 0.41	1.28 ± 0.47	−1.00 ± 0.28	0.09 ± 0.29	0.89 ± 0.50	1.43 ± 0.54		

[a] Uncertainties represent 2SD (‰) for 3−10 replicates.

Isotopic Composition of Synthesized Dimethylmercury (Me_2Hg). As a second application of the LRS method, we measured the isotopic composition of Me_2Hg species. Dimethylmercury was synthesized from $HgCl_2$ by methylation using the Grignard reaction.[18] The yield recovery of the synthesis was found to be about 83% for Me_2Hg, and isotopic ratios measured for this species are presented in Table 4. Synthesized Me_2Hg has a mass-dependent isotopic composition relative to NIST-3133, with $\delta^{202}Hg = -0.80 \pm 0.26$. Also isotopic composition was measured for the initial salt of $HgCl_2$ (Merck), from which Me_2Hg was synthesized, relative NIST-3133 by CV-MC-ICPMS. $\delta^{202}Hg$ was found to be -0.91 ± 0.29‰ having a mass-dependency for all Hg isotopes. Hence, no fractionation was observed during Grignard dimethylation of IHg^{2+} with the yield of 80−90%.

It is important to note that sometimes the elution peak of the species is located not far from the elution of the solvent. For example, Hg^0 and Me_2Hg species are eluted just before and after hexane, respectively (Figure 5). In addition, elution of hexane disturbs signals of analyte isotopes, such as Tl and Hg, leading to inaccurate isotopic ratios results during this elution. In this case, it is important to choose proper peak zones of species for the isotopic ratio calculation using the LRS method. It is necessary to avoid crossing of the species peak with the signal disturbance. For example, in the case of Me_2Hg, the peak zone should be started just before the beginning of elution, and in the case of Hg^0, the peak zone should be ended just after the finishing of elution (Figure 5).

Isotopic Composition of Biological Reference Materials. Another application of the method which is demonstrated in Table 4 is the measurement of the isotopic composition in the species of environmental and biological samples. The isotopic composition in species of tuna fish (ERM-CE464) and human hair (IAEA-085 and IAEA-086) were measured using GC-MC-ICPMS. Isotopic ratios for both species of IHg^{2+} and MeHg were calculated using the LRS method relative to the NIST-3133 bracketing standard.

Reference material of human hair IAEA-085 represents hair with an elevated level of methylmercury: MeHg, 22.9 mg kg^{-1} and IHg^{2+}, 0.3 mg kg^{-1}. Since this CRM was spiked with a high concentration of methylmercury, the isotopic composition of this material represents the isotopic composition of the spiked solution. As can be seen from Table 4, both MeHg and IHg^{2+} have mass-dependent isotopic composition for all Hg isotopes, since $\Delta^{xxx}Hg$ values are statistically equal to zero. Isotopic composition of IHg^{2+} species ($\delta^{202}Hg = -0.31 \pm 0.12$‰) in this CRM is slightly more positive than MeHg ($\delta^{202}Hg = -0.48 \pm 0.24$‰). The total isotopic composition calculated using mass-balance of the species is in a good agreement with previously published values.[3,20] For example, the total $\delta^{202}Hg = -0.48 \pm 0.23$‰ using the LRS method, while it is -0.37 ± 0.15‰ for total Hg in Laffont et al.[20] and -0.37 ± 0.41‰ for MeHg in Epov et al.[3]

The reference material of human hair, IAEA-086, represents cryogenically homogenized hair without any additions of Hg, having following concentrations of Hg species: MeHg, 0.258 mg

(20) Laffont, L.; Sonke, J. E.; Maurice, L.; Hintelmann, H.; Pouilly, M.; Sanchez Bacarreza, Y. S.; Perez, T.; Behra, P. *Environ. Sci. Technol.* **2009**, *43* (23), 8985–8990.

kg^{-1}, and IHg^{2+}, 0.315 mg kg^{-1}. As can be seen from Table 4, MeHg species in this CRM has a significantly positive mass-dependent isotopic composition for even Hg isotopes ($\delta^{202}Hg = +1.70 \pm 0.24‰$). Also, positive mass-independent isotopic fractionation was observed for the odd Hg isotopes ($\Delta^{201}Hg = +0.49 \pm 0.09‰$ and $\Delta^{199}Hg = +0.67 \pm 0.01‰$). The ratio of $\Delta^{199}Hg/\Delta^{201}Hg$ is equal to 1.37 ± 0.22, which corresponds to the ratio obtained by the photochemical reactions for MeHg.[15] Species of IHg^{2+} in this CRM has less positive mass-dependent isotopic composition for the even Hg isotopes ($\delta^{202}Hg = +0.25 \pm 0.23‰$) and slightly negative mass-independent isotopic fractionation for the odd Hg isotopes ($\Delta^{201}Hg = -0.15 \pm 0.11‰$ and $\Delta^{199}Hg = -0.17 \pm 0.11‰$). The ratio of $\Delta^{199}Hg/\Delta^{201}Hg$ is equal to 1.13 ± 0.23, which is close to the ratio obtained by the photochemical reactions for IHg^{2+}.[15]

Reference material of tuna fish, ERM-CE464, represents homogenized fish with the natural level of Hg species: MeHg, 5.50 mg kg^{-1} and IHg^{2+}, 0.132 mg kg^{-1}. As can be seen from Table 4, MeHg species in this CRM has a positive mass-dependent isotopic composition for the even Hg isotopes ($\delta^{202}Hg = +0.55 \pm 0.24‰$) and strong mass-independent isotopic fractionation for the odd Hg isotopes ($\Delta^{201}Hg = +1.97 \pm 0.26‰$ and $\Delta^{199}Hg = +2.41 \pm 0.15‰$). These values are in a good agreement with previously published results.[3,20] Species of IHg^{2+} in this CRM have strongly negative mass-dependent isotopic fractionation for the even Hg isotopes ($\delta^{202}Hg = -2.18 \pm 0.38‰$) and also strong positive mass-independent isotopic fractionation for the odd Hg isotopes ($\Delta^{201}Hg = 1.28 \pm 0.47‰$ and $\Delta^{199}Hg = 1.43 \pm 0.54‰$). The ratio of $\Delta^{199}Hg/\Delta^{201}Hg$ is equal to 1.23 ± 0.13 and 1.12 ± 0.04 for MeHg and IHg^{2+}, respectively. These ratios are in a good agreement with previously published results for the photochemical reactions.[15]

CONCLUSIONS

A simple and precise method to measure isotopic composition (δ values) of different elemental species of Hg using GC-MC-ICPMS has been presented. The best external precision and accuracy of the method was observed when the concentration of the species in a sample and in a standard is high and similar. However the method can be also used for different concentrations of Hg species in the sample and the standard. Also, with the use of this method, the isotopic composition of several Hg species presented in the sample can be measured relative to single species presented in the bracketing standard. In addition, the methodology allows studying the isotopic composition of low abundance isotopes. In some cases, the new method of data reduction is advantageous over the continuous signal data.

As an application of the new methodology for various environmental and biological samples, such as fish tissue and human hair, we have demonstrated the results of $\delta^{204}Hg$, $\delta^{202}Hg$, $\delta^{201}Hg$, $\delta^{200}Hg$, $\delta^{199}Hg$, and sometimes $\delta^{196}Hg$ in Hg^0, Me_2Hg, MeHg, and IHg^{2+} species. Also, isotopic fractionation during degradation of IHg^{2+} to Hg^0 was measured in both species. Results demonstrate the mass-dependent and mass-independent fractionation of Hg isotopes in different environmental processes and samples.

This method could be a revolutionary new approach to study the isotopic composition of species, since it can be applied for other hyphenations of chromatographic techniques with MC-ICPMS, such as using GC-MC-ICPMS, HPLC-MC-ICPMS, or LC-MC-ICPMS.

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