MEASUREMENT OF COMPOUND-SPECIFIC Hg ISOTOPIC COMPOSITION IN NARROW TRANSIENT SIGNALS BY GAS CHROMATOGRAPHY COUPLED TO MULTICOLLECTOR ICP-MS

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Abstract

The study of the natural variations of Hg isotopic composition is a valuable tool to understand Hg biogeochemical cycle. Hg mobility, toxicity and bioaccumulation depends on its chemical form, so compound-specific Hg isotope ratio measurements, by coupling chromatographic techniques to multicollector instruments, provide an extra degree of information in comparison to total Hg isotope ratio measurements. We present in this work a thorough evaluation of the main parameters affecting the accuracy and precision of Hg compound-specific isotope ratio measurements by Gas chromatography (GC) coupled to MC-ICPMS. The main parameters evaluated in this work were the chromatographic peak width, integration time, number of acquisition points and data treatment strategy. A new method for the correction of the time lag between Faraday cups, responsible of isotope ratio drift during peak elution, is proposed and evaluated under different experimental conditions. A standard-sample-standard bracketing approach was applied to calculate the delta values from the mass bias corrected Hg(II)-specific isotope ratios, using NIST-3133 as delta zero standard. The optimized conditions were obtained when working with regular GC peaks (2-5 s at the peak base), 0.131 s as integration time, 321-641 acquisition points and calculating the isotope ratios using the slope of the linear regression obtained when plotting two isotopic signals. The accuracy and precision of the optimized methodology were determined with the analysis of the secondary standard NIST RM-8610 (UM-Almaden) versus the delta zero standard NIST-3133. When injecting 2 µL of a Hg(II) solution of 250 ng g^{-1} and Hg(II)-specific delta values in agreement with the reference values were obtained with an external reproducibility (expressed as 2SD) of 0.34 -0.40 ‰. Our results demonstrate that the measurement of Hg isotope ratios in narrow GC peaks provides the same level of accuracy and external reproducibility of Hg compound specific delta values in comparison with previously published approaches based on wide GC peaks. Therefore offering lower analysis times and higher chromatographic resolution than those previously obtained with wide chromatographic peaks

1. INTRODUCTION

Mercury is a global pollutant released to the atmosphere by natural and anthropogenic processes[1], and occurs in different chemical forms and/or oxidation states in terrestrial, atmospheric and aquatic ecosystems.[2] On one hand, the determination of the different Hg species in a sample (speciation analysis) can be extremely helpful to understand Hg biogeochemical cycle as Hg reactivity, mobility and bioaccumulation depend on its chemical form. On the other hand, the accurate and precise measurement of Hg isotopic composition in environmental samples is a valuable tool to understand Hg pathway in the environment and to fingerprint contamination sources.[3,4] Hg has seven stable isotopes that can undergo mass-dependent and/or mass-independent fractionation (MDF and MIF, respectively) during different bio-geochemical processes. MIF of Hg isotopes have been related to two mechanisms: the magnetic isotope effect and the nuclear volume effect (also known as nuclear field shift effect),[5–9] MIF of Hg isotopes is preserved in many transformations and can be related to the provenance of mercury.[10]

The coupling of chromatographic techniques to multicollector instruments to measure compound-specific Hg isotopic compositions may lead to new insights into the biogeochemical behavior of mercury species in the environment. Most of the studies carried out thus far on the fractionation of Hg isotopes are focused on total Hg isotope signatures[11–13] and only a few publications have measured Hg species-specific isotopic compositions.[14–17] This is mainly due to the difficulties encountered in the measurement of isotope ratios in transient signals and the low concentration levels of Hg species in real samples.[18] Exhaustive sample preparation procedures including preconcentration steps[19] or alternative protocols for the selective extraction of MeHg have been proposed.[20]

In 2001, Krupp and coworkers[21] reported the first application on the hyphenation of a chromatographic technique to a MC-ICPMS reporting precisions in lead isotope ratio measurements ranging from 0.008 to 0.2% of RSD for tetraethylated lead. In 2008, Epov[14] and coworkers reported an external reproducibility of 0.56‰ for δ^{202}Hg as 2SD, obtained by GC-MC-ICP-MS. This approach was mainly based on a GC-adapted standard-sample-standard bracketing scheme and the widening of the chromatographic peak using isothermal temperature programs to increase the number of acquisition points during the chromatographic peak elution.

Concerning data treatment, the accuracy and precision of compound-specific isotope ratios was improved by calculating the isotopic ratios from the slope of a linear regression between isotopic signals. This strategy was initially developed by Fietzke *et al*[22] for transient signals obtained by Laser Ablation coupled to MC-ICP-MS and lately applied by Epov *et al*[23] to measure compound-specific isotope ratios of Hg by GC-MC-ICP-MS. The external reproducibility (2SD) obtained applying this strategy ranged between 0.2-0.5‰ for δ^{202}Hg. Using this approach the data treatment procedure is significantly simplified as the selection of a specific percentage of acquisition points within the chromatographic peak is avoided and the background correction is straightforward.

Isotope ratio drift during peak elution has been reported when hyphenating a chromatographic technique to MC-ICPMS. Initially, this was attributed to isotopic fractionation during sample introduction or chromatographic separation[21,24,25,26] but further studies demonstrated that the drift is due to the Faraday cups desynchronization. [27,28] This phenomenon has been explained by the slow time response of the amplifier system which can vary between individual collectors when measuring fast changes in input ion currets.[29] Therefore, several methods based on the calculation of the time lag between different amplifier responses were proposed to correct for isotope ratios drift during transient signals when coupling either Laser Ablation or GC to a MC-ICP-MS.[29,30]

We present in this work an evaluation of the main parameters affecting the accuracy and precision of the measurement of compound-specific isotope ratios of Hg by GC-MC-ICPMS: chromatographic peak width, integration time, number of acquisition points, data treatment strategy and isotope ratio drift correction during peak elution.. The accuracy and precision of the optimized methodology were determined with the analysis of the secondary standard NIST RM-8610.

2. MATERIALS AND METHODS.

2.1 Reagents and materials

The standard reference materials NIST RM-8610 (UM-Almaden) and NIST SRM-3133 were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Thallium standard solution was purchased from Absolute Standards Inc. (Hamden, CT, USA). The Tl standard was diluted in 3% ultrapure sub-boiled HCl in Milli-Q water (≥18 MΩ cm). An acetic acid/sodium acetate buffer (0.1M, pH 4) was prepared by dissolving sodium acetate and acetic acid (Sigma-Aldrich) in Milli-Q water and adjusting to pH 4. The ethylation of inorganic mercury was performed using a 2% (w/v) solution of sodium (tetra-n-ethyl)borate (LGC-Standards, Wesel, Germany) in Milli-Q water. Hexane (Sigma-Aldrich) was employed for the extraction of the derivatised mercury compound.

2.2 Sample preparation for isotope ratio measurements

Working solutions of Hg(II) were prepared in 7 mL glass vials containing 4 mL of acetic acid/acetate buffer (0.1M, pH 4) by the addition of the appropriate amount of the standard solution to obtain a final concentration of 250 ng (of Hg) g^{-1} in the final organic phase. The mercury species was ethylated and extracted into an organic phase by the addition of 1 mL of hexane and 0.200 mL of sodium(tetra-n-ethyl) borate 2%, followed by 5 minutes of manual shaking. Finally, the organic phase was transferred to a 2 mL glass vial.

2.3 Instrumentation

A gas chromatograph model Agilent 6890N (Agilent Technologies, Tokyo, Japan) fitted with a split/splitless injector and a DB-5MS capillary column from Agilent J&W Scientific (cross-linked 5% diphenyl, 95% dimethylsiloxane, 30 m × 0.53 mm i.d. × 1.0 μm) was coupled to a Multicollector Inductively Coupled Plasma Mass Spectrometer Neptune Plus (ThermoScientific, Bremen, Germany). The GC-MC-ICP-MS interface consisted of a heated metallic block which enables the mixing of the Ar gas flow coming from the MC-ICPMS (sample gas) with the carrier gas of the GC Column to transfer the eluted Hg species into the ICP source (**Figure 1**). A cyclonic spray chamber and a PFA concentric nebulizer working at 700 μL min^{-1} were coupled to the GC-ICP-MS system through a T-piece before the ICP source, allowing the introduction of a wet

aerosol of a Tl solution for mass bias correction. Thus, the nebulizing gas is used for the nebulization of the Tl solution and the sampling gas is used for the transfer of the Hg species eluted from the GC column. The GC-MC-ICP-MS instrumental parameters are summarized in **Table 1**. All samples and standards were weighted on an analytical balance Metler Toledo MS Semi-micro MA.205DU (0.0001 g). The pH was adjusted with a Basic 20 CRISON pH-meter (Alella, Barcelona, Spain).

2.4 Measurement of compound-specific Hg isotope ratios

The isotopes ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{203}Tl, and ^{205}Tl were simultaneously measured in the Faraday cups L3, L2, L1, C, H1, H2, and H3, respectively. All measurements were performed in a static multicollection mode using the conventional acquisition software of the instrument. The isotopes ^{204}Hg and ^{196}Hg could not be measured due to the specific Faraday cups configuration of our MC-ICP-MS instrument. ^{203}Tl and ^{205}Tl were continuously measured for mass bias correction nebulizing a Tl solution in 3% HCl. The chromatographic conditions applied in this work are given in **Table 1**. When measuring Hg isotope ratios in narrow GC chromatographic peaks (2-5 seconds at the peak base) integration times of 0.131 or 0.262 s were evaluated and the separation of Hg species was achieved in 6.75 minutes. When measuring Hg isotope ratios in wide chromatographic peaks, integration times of 0.524 and 1.049 s were evaluated. When measuring only Hg(II) isotope ratios in wide peaks, a total analysis time of 13.1 minutes was required for each GC run. It is worth noting that for the calculation of a delta value three GC runs are required. If both MeHg and Hg(II) isotope ratios needed to be measured using wide peaks the analysis time of each GC run increased to 19.1 min. Before starting any measurement session, the Faraday-amplifier gains were calibrated and the mass window, lenses, torch position and Ar flows were optimized using the ^{205}Tl signal. The configuration of the Faraday cups was initially performed by nebulisation of a Hg standard solution of 20 ng g^{-1}. However, in order to avoid Hg contamination in the MC-ICP-MS system due to memory effects, mass accuracy was checked and adjusted daily with the measurement of a 20 ng g^{-1} Tl solution

2.5 Data reduction

Three different approaches for the calculation of compound-specific Hg isotope ratios were compared in this work: Peak Area Integration (PAI), Linear Regression Slope (LRS)[22,23] and Point by Point (PbP).

2.5.1 Calculation of isotope ratios by peak area integration

Using the PAI method the compound-specific isotope ratio was calculated dividing the sum of the background corrected voltages obtained for each isotope in a selected range of the chromatographic peak as described elsewhere.[16]

2.5.2 Calculation of isotope ratios by linear regression slope

The LRS method calculates the isotope ratio as the slope *b* of a linear fit of a selected range of the isotopic intensities as described in equation (1) where V^{xxx} is the voltage obtained for the isotope xxx, being xxx the mass of the isotopes between ^{199}Hg and ^{204}Hg, and *a* is the intercept.

$$V^{xxx} = V^{198} \cdot b + a \qquad (1)$$

According to the recommendations proposed by Blum and Bergquist[31] for reporting variations in the natural isotopic composition of Hg, the internal precision of an individual analysis should be reported as 2 times the standard error of the mean, which is equivalent to the standard error of the slope using the LRS method[32]. Using the LRS method the internal precision was evaluated using the standard error of the slope as described in equation 2 (adapted from Miller and Miller[33]):

$$u_{LRS} = \frac{\sqrt{\frac{\sum\left(V_{xxx_i} - \hat{V}_{xxx_i}\right)^2}{n-2}}}{\sqrt{\sum\left(V_{198_i} - \overline{V}_{198}\right)^2}} \qquad (2)$$

Note that V_{xxx_i} is the measured voltage and $\hat{V}_{xxx_i}$ the predicted voltage by the linear regression for the isotope xxx in each acquisition point. Therefore, $\sum\left(V_{xxx_i} - \hat{V}_{xxx_i}\right)^2$ corresponds to the squared sum of residuals and n is the number of acquisition points used to define the regression line. V_{198_i} is the measured voltage for the isotope 198 in each acquisition point and $\overline{V}_{198}$ the average voltage of the selected range of acquisition points. From a practical point of view, both the slope and the standard error

of the slope were calculated using the function LINEST in Microsoft Excel. After Peak apex identification using spreadsheet software, an optimized number of 160 acquisition points were selected both before and after the apex leading to a total number of acquisition points of 321.

2.5.3 Calculation of isotope ratios point by point

When applying the PbP method, the isotope ratio is calculated in each acquisition point using background corrected voltages. Then, the average of the isotope ratios measured over a certain range of acquisition points within the transient signal is calculated. In contrast to the LRS method, baseline points of the chromatogram are not included in the selected range. Thus a background correction of the voltage is required. The number of acquisition points within the chromatographic peak ranged from 1 (peak apex) to 21 points. To evaluate the internal precision of the PbP method, the standard error of the mean was calculated using equation (3) (adapted from Miller and Miller[33]):

$$u_{PbP} = \sqrt{\frac{n}{n-1}\sum\left(\left(\frac{V_{xxx}}{V_{198}}\right)_i - \left(\overline{\frac{V_{xxx}}{V_{198}}}\right)\right)^2} \qquad (3)$$

2.5.4 Correction of the time-lag between Faraday Cups

The correction model applied in this work assumes that the source of the isotope ratio drift during peak elution is the non-uniform time response of the different amplifiers. Similar to Gourgiotis *et al*,[30] our correction model is based in the assumption that the cup L3 (m/z=198) is the reference on time while the other four cups (L2, L1, C and H1) are delayed. As the time-lag affects mostly the internal precision of the isotope ratio measurement in transient signals, cups H2 and H3, used for ^{203}Tl and ^{205}Tl, respectively, are not included in this model. **Figure S1** in the Supporting information shows that, if the variation of the signal during the time-lag interval, $V_2^{xxx} - V_{corr}^{xxx}$ can be assumed to be linear and proportional to that observed during the integration time, $V_2^{xxx} - V_1^{xxx}$, the slopes of both lines will be the same and those can be described in equation (4) as;:

$$\frac{V_2^{xxx} - V_1^{xxx}}{\Delta t_i} = \frac{V_2^{xxx} - V_{corr}^{xxx}}{\Delta t} \quad (4)$$

where Δt_i is the integration time used in the isotope ratio and Δt is the time-lag between the corresponding Faraday cup and the L3 cup. Then, we can calculate the corrected voltage V_{corr} using equations (5) and (6).

$$V_{corr}{}^{xxx} = V_2{}^{xxx} - \frac{\Delta t}{\Delta t_i} \cdot \left(V_2{}^{xxx} - V_1{}^{xxx}\right) \quad (5)$$

In these equations V_1 and V_2 are the voltages measured for the isotope *xxx* by the delayed cup at the beginning and at the end of the integration time (Δt_i). Note that the calculation of V_{corr} by equation (6) requires the previous calculation of the time-lag (Δt) for each cup. If we consider that the time-lag is the only factor affecting the isotope ratio drift, the Δt value can be obtained mathematically. In this work we have calculated the time lag for each cup by minimizing the squared sum of residuals of the linear regression applied to calculate the isotope ratio. The minimization of the residuals directly leads to the minimization of the standard error of the slope or the maximization of the r^2 of the linear regression as done in Claverie *et al*[34] This can be performed using spreadsheet calculation software such as the Excel Solver function avoiding the restriction to negative variables. In the case of PbP method, the Δt value can be obtained iteratively by minimizing the standard error of the mean given in equation (3). This last method is analogue to the minimization of the drift slope as done in Gourgiotis *et al*[30]. A comparison of the different correction methods used in the literature for the LRS calculation is given in **Figure S2** in the Supporting Information. As can be observed, the final time lag results are equivalent regardless of the calculation method used.

2.5.5. Mass bias correction

The elution of the sample matrix from the GC column may induce plasma instabilities affecting mass bias during the chromatographic peak profile of Hg compounds. To minimise this problem a Tl solution was simultaneously nebulized into the ICP source using a microconcentric nebulizer and a cyclonic spray chamber. The Tl spray was mixed through a T piece with the Ar flow transporting the gaseous analytes as described in **Figure 1**. Such "wet" plasma conditions prevent the accumulation of carbon particles in the ICP cones due to the combustion of the organic solvent.[14,20]The concentration of

the Tl solution was selected to match the ^{205}Tl voltage and the peak apex voltage of ^{202}Hg within 10%[14]. Mass bias was then corrected applying the Russel equation as described in equation (7):

$$\left(\frac{^{xxx}Hg}{^{198}Hg}\right)_{corrected} = \frac{\left(\frac{^{xxx}Hg}{^{198}Hg}\right)_{measured}}{\left(\frac{W^{xxx}}{W^{198}}\right)^{f}} \quad (7)$$

W is the isotopic mass of the corresponding isotope and f is the correction factor calculated using equation (8):

$$f = \log\left(\frac{\left(\frac{^{205}Tl}{^{203}Tl}\right)_{measured}}{\left(\frac{^{205}Tl}{^{203}Tl}\right)_{theoretical}}\right) \quad (8)$$

Measured Tl isotope ratios were calculated by the LRS procedure (intercept set to zero) within the range of acquisition points selected for the measurement of compound-specific isotope ratios. When working with continuous signals both the PbP and the LRS procedure provide the same values of isotope ratios and uncertainties[32].

2.5.6 Calculation of Hg species-specific delta values

Hg species-specific delta values by GC-MC-ICP-MS were calculated using equation (9) after applying a standard-sample-standard bracketing approach using the standard reference material NIST-3133 as delta zero standard.

$$\delta^{xxx}Hg = \left(\frac{\left(\frac{^{xxx}Hg}{^{198}Hg}\right)_{sample}}{\left(\frac{^{xxx}Hg}{^{198}Hg}\right)_{standard}} - 1\right) \times 1000 \quad (9)$$

In equation (9), xxx refers to the mass of the isotopes between ^{199}Hg and ^{202}Hg. The isotope ratio of the standard NIST-3133 is calculated from the average of the isotope ratio measurement before and after the sample. The concentration of the standard was adjusted to match the intensity of that of the sample within 10%[14]. Capital delta values representing MIF were calculated using equation (10) where β_{xxx} is the kinetic mass-

dependence scale factor for each isotope: 0.2520 for ^{199}Hg, 0.5024 for ^{200}Hg, and 0.7520 for ^{201}Hg.[31]

$$\Delta xxx_{Hg} = \delta^{xxx}Hg - (\delta^{202}Hg \times \beta_{xxx}) \quad (10)$$

3. RESULTS AND DISCUSSION

3.1. Optimization of Ar flows for compound-specific isotope ratio measurements

Our GC-MC-ICP-MS instrument requires the use of two Ar flows which are mixed before entering into the ICP source through a "t" piece. The nebulizing gas is used for the nebulization of the Tl solution and the sampling gas is used for the transfer of the Hg species eluted from the GC column. The optimization of both flows is critical to achieve the maximum sensitivity. **Figure 2** shows the voltages obtained for ^{205}Tl and ^{202}Hg when nebulizing a Tl solution of 20 ng g^{-1} and injecting 2 µL of a Hg(II) solution of 250 ng g^{-1} at different flow rates of nebulizing and sampling gas. As the chromatographic peak width is not significantly affected by the Ar flows, Figure 2 shows the Hg(II) peak height (V) instead of peak area. Under all conditions a total flow of 1.2 L min^{-1} was maintained and the sample gas was increased from 0.2 to 0.8 L min^{-1}. As observed in **Figure 2**, the highest ^{202}Hg(II) signal was obtained with a sample gas flow of 0.7 L/min and a nebulizing flow of 0.5 L/min. The Ar flows were optimised with the Tl signal on a daily basis and we found minimal variations in the optimal values.

3.2. Optimization of the chromatographic conditions for compound-specific isotope ratio measurements

Optimum GC separations provide very short transient signals (typically 2-5 s at the peak base).Therefore, the number of acquisition points during the chromatographic peak with high and measurable signals is very small and generally not enough to obtain comparable levels of precision than those obtained when measuring continuous signals at the same voltage levels. Intentional broadening of the chromatographic peaks[14] has been applied in previous works to increase the number of acquisition points to simulate a continuous signal. The use of isothermal temperature programs has been reported for

the measurement of compound specific Hg isotope ratios in peaks of 30-60 s width at the peak base.[14,23]

Initially, we have also applied isothermal temperature programs to obtain the maximum peak width maintaining the voltage higher than 0.5 V for ^{202}Hg at the top of the peak.[14] If Hg isotope ratios are measured for MeHg and Hg(II) in the same sample, the temperature program must include two isothermals of several minutes to elute both species. After a careful optimization we found that one isothermal at 45°C for MeHg and another at 63°C for Hg(II) both for 7 minutes were required to obtain the desired peak broadening. The chromatographic profile for Hg(II) obtained using these conditions is shown in **Figure 3b**. As can be observed, chromatographic peaks of 30-35 s at the peak base are obtained using T program 2. However, **Figure 3c** shows that when applying only one isothermal the peak width of Hg(II) decreases to 20-25 s at the peak base. A total acquisition time of 19.1 min was required to separate both Hg species in a sample but the total analysis time decrease to 13.1 minutes if only Hg(II)-specific isotope ratios are measured. Due to the lack of a delta zero standard for MeHg the optimization of the isotope ratio measurements by GC-MC-ICP-MS was carried out only for Hg(II). Therefore, the GC temperature program 3 given in Table 1 was applied when working with wide chromatographic peaks throughput this work to save time during the measurement sessions.

In order to reduce the analysis time and increase the sensitivity and the chromatographic resolution, we also optimized the measurement of compound-specific Hg isotope ratios in regular GC peaks. Therefore, a conventional GC temperature program employed previously for the simultaneous determination of MeHg, EtHg and Hg(II) by isotope dilution[35] was also applied in the optimizations. As can be observed in **Figure 3a**, the GC temperature program 1 (**Table 1**) provides transient signals of 2-5 s at the peak base. **Figure S3** in the Supporting Information compares two chromatograms of a standard solution containing 250 ng g^{-1} of Hg(II) obtained with T program 1 and T program 3. As can be observed the elution of Hg(II) is achieved in 3.5 minutes under T program 1. The concentration of the nebulized Tl solution was adjusted to obtain a similar voltage than that obtained at the top of the Hg(II) peak. As expected, when working with an isothermal T program the peak height was significantly decreased in T program 3 in comparison with T program 1. As observed in **Figure** S3, the voltage at the top of the peak decreased from 1.75 to 0.65 V with increasing the retention time and

peak width. We can also observed in both chromatograms the distortion of the Tl signals when eluting the hexane from the column and a small peak of Hg(0) before hexane elution.[35] Hg(0) peak can be due to a contamination problem or the result of Hg(II) reduction during sample preparation. In the latter case this can be a source of isotopic fractionation but in all cases the voltage of the Hg(0) peak was negligible in comparison with Hg(II). Both temperature programs 1 and 3 will be compared in subsequent studies.

3.3. Correction of the time-lag between Faraday cups for compound-specific isotope ratios measurements

Although MC-ICP-MS instruments are designed to allow the simultaneous measurement of isotopic signals, many authors have reported isotope ratio drifts during peak elution. This has been attributed to differences on the time response of the Faraday cups amplifiers when measuring fast changes in input ion currents rather than to chromatographic isotope effects.[28] Therefore, the correction of the time lag between cups is expected to improve the internal precision of the isotope ratio measurements. Several methods have been applied to calculate the time lag between different amplifier systems and correct for the isotope ratio drift during transient signals.[28-30]. We assume a linear variation of the signal during the time-lag interval proportional to the variation during the integration time (equation 4). We also assume that the time-lag (Δt) is the only factor affecting the IR drift during peak elution. Under these conditions, using the LRS method, Δt was obtained iteratively by minimizing the squared sum of residuals of the linear regression applied to calculate the isotope ratio. As demonstrated in Figure S2, this method is equivalent to that proposed by Claverie *et al*[34] which maximized the squared correlation coefficient of the linear regression. Using the PbP method, Δt was obtained iteratively by minimizing the standard error of the mean given in equation (3) which is equivalent to the method of Gourgiotis *et al*[30] which minimized the drift slope.

Table S1 in the Supporting Information shows the Δt values obtained in three different measurement sessions in which we carried out n=17 independent GC-MC-ICPMS injections of 2 µL of a solution containing 250 ng g^{-1} of Hg(II). In all measurements, the GC T program 1 (narrow peaks) and the LRS were applied and the Hg(II)-specific isotope ratios were measured using an integration time of 0.131 s and 321 acquisition

379 points. After peak apex identification, 160 acquisition points were selected before and
380 after the apex leading to a total number of acquisition points of 321The values obtained
381 for cups L2, L1, C and H1 were 2.8 ± 0.2, 1.8 ± 0.2, 3.4 ± 0.2 and 2.0 ± 0.2 ms,
382 respectively. The time lags shown in **Table S1** are significantly lower than the
383 integration times evaluated in this work except for the case of the shortest integration
384 time of 0.131 s. Thus, isotope ratio drift is expected to be less pronounced for longer
385 integration times (e.g. 1 s or longer). **Figure 4** shows a representative chromatographic
386 peak profile and the isotope ratio obtained using 0.131 s (Figure 4a) and 0.262 s (Figure
387 4b) for the narrow GC peak, and using 0.524 s (Figure 4c) and 1.049 s (Figure 4d) for
388 the wide GC peaks. As can be observed in Figure 4, the proposed method corrects for
389 isotope ratio drifts which are more pronounced for lower integration times. The average
390 values of Table S1 were used for correction in further measurements to reduce the
391 complexity of the data treatment.

392 **Figure 5** shows the comparison of the internal precision of time-lag corrected and
393 uncorrected Hg(II)-specific $^{202}Hg/^{198}Hg$ isotope ratio both applying the LRS and the
394 PbP methods in narrow and wide GC peaks obtained for n=17 independent replicates
395 measured under different conditions. **Figure 5a** shows the results obtained applying the
396 LRS method in narrow GC peaks using 0.131 s of integration time and 21, 81, 161 and
397 481 acquisition points while **Figure 5b** shows the results obtained using wide GC peaks
398 and 1.049 s of integration time for 21 and 161 acquisition points. **Table S2** shows the
399 raw data for 202/198 isotope ratios and internal precisions (as 2SE) obtained under all
400 conditions assayed using the LRS method. Alternatively, **Figure 5c** shows the results
401 obtained applying the PbP method in narrow peaks with 0.131 s integration time and 5
402 acquisition points while **Figure 5d** shows those obtained in wide peaks, 1.049 s
403 integration time and 5 acquisition points. As can be observed, when applying the LRS
404 method and working with narrow GC peaks and 0.131 s integration time (Figure 5a),
405 the time lag correction improves the internal precision of the Hg(II)-specific
406 $^{202}Hg/^{198}Hg$ isotope ratios in all cases, but the effect is more pronounced when a lower
407 number of acquisition points are considered (21-161). For a number of 481 acquisition
408 points corrected and uncorrected isotope ratios show similar internal precisions. When
409 applying the LRS method in wide peaks and 1.049 s integration time, there was not a
410 significant difference between the internal precision of corrected or uncorrected isotope
411 ratios (**Table S2**). When applying the PbP method, the internal precision of the isotope

ratios improves with the time lag correction only when using 0.131 s as integration time. Those results confirm that the proposed time lag correction improves the internal precision of isotope ratios when working with low integration times. Also, as observed in Figure 4, the isotope ratio drift is less pronounced with higher integration times and wider GC peak profiles.

3.4. Evaluation of the accuracy and precision of Hg species-specific delta values.

In order to optimize the accuracy and precision of Hg-species-specific delta values by GC-MC-ICP-MS we evaluated the following parameters: i) calculation method, ii) number of acquisition points iii) GC peak width and iv) integration time. To facilitate the standardization and intercomparison of our results with other laboratories, delta values and their associate uncertainties were calculated following the suggestions proposed by Blum and Bergquist,[31] for reporting variations in the natural isotopic composition of Hg. The external precision or reproducibility of our methodology was thus expressed as 2 times the standard deviation (2SD) of several measurements of delta values versus the delta zero standard NIST-3133. According to previous works in this field[14,23], an acceptable external precision for compound-specific delta values should be lower than 0.5 ‰ as 2SD.

The selection of an optimal range of acquisition points has been carried out in previous works[14,23] selecting different chromatographic peak percentages. However, in order to facilitate the comparison between the different data treatment strategies applied in this work, we have evaluated the number of acquisition points of the chromatogram rather than peak percentages. Three different data reduction strategies were evaluated in this work: Linear Regression Slope (LRS), Point by Point ratio measurements (PbP) and Peak Area Integration (PAI). The three methods were applied to calculate δ values in narrow GC peaks (2-5 s) and wide GC peaks (20-25 s). In addition, different integration times were evaluated for each peak profile. When working with narrow GC peaks, the measurements were performed with shorter integration times (0.131 s and 0.262 s) whereas longer integration times of 0.524 and 1.049 s were used when working with wider peaks as recommended previously.[14]

3.4.1 Analysis of NIST-3133 vs NIST-3133

The first optimizations were carried out calculating the δ values of NIST-3133 bracketed with the same NIST-3133. This study was carried out in four different days performing in each session n=17 independent GC-MC-ICPMS injections of 2 µL of a solution containing 250 ng g^{-1} of Hg(II). The first session was performed applying GC program 1 (narrow peak) and 0.131 s integration time, the second applying the GC program 1 (narrow peak) and 0.262 s integration time, the third applying the GC program 3 (wide peak) and 0.524 s integration time and the last session applying GC program 3 (wide peak) and 1.049 s integration time. **Figure 6** shows the average of δ^{202}Hg(II) (‰), and its associated external precision (±2SD) represented as error bars for n=8 independent measurements under the different experimental conditions assayed. **Figure S4** of the supporting information shows the results obtained for δ^{199}Hg(II), δ^{200}Hg(II) and δ^{201}Hg(II) and **Table S3** of the supporting information shows the raw data obtained for all delta values and their associated external precisions in all measurement sessions.

Our results show that the best external precision was obtained using the LRS method working with narrow GC peaks, 0.131 s of integration time and selecting between 321 and 641 acquisition points. Under these conditions the 2SD of all delta values ranged from 0.236 to 0.590 ‰. These results demonstrate the importance of including enough number of acquisition points from the background when working with the LRS method. .When using 0.131 s and a lower number of acquisition points, the 2SD values increased significantly (from 0.420 to 0.874 ‰ for 81 acquisition points and from 0.575 to 1.291 ‰ for 21 acquisition points). A worse external reproducibility using the LRS method was also obtained when working with wide chromatographic peaks as the best 2SD values ranged from 0.341 to 0.591 ‰ with 81 acquisition points. Using the PAI method, the best external precision was obtained with narrow GC peaks, 0.131 s of integration time and 41 or 81 acquisition points. Using these conditions, the 2SD of the delta values ranged from 0.433 to 0.641 ‰. Worse 2SD values were obtained using PAI and wide chromatographic peaks as the best 2SD for the delta values ranged from 0.508 to 1.107 ‰ for 1.049 s integration time and 21 acquisition points. Finally, when using the PbP method the external precisions obtained using 0.131 s integration time, narrow peaks and 21 acquisition points (0.547-0.845 ‰) were comparable to those obtained with wide peaks, 1.049 s of integration time and 5 acquisition points (0.485-0.885 ‰).

Previous works on the measurement of Hg isotope ratios in transient signals applied isothermal temperature programs to increase the chromatographic peak width and hence the number of acquisition points. Peak area integration was initially applied as data reduction strategy obtaining an external 2SD precision of 0.56 ‰ for $\delta^{202}Hg$[14]. More recently, it was demonstrated that improved precision and accuracy was obtained applying LRS with wide chromatographic peaks (0.2-0.5‰ for $\delta^{202}Hg$)[23]. However, as shown in Table 1 the use of isothermal GC temperature programs requires a significantly longer chromatographic run than those applied in conventional GC separations. In addition, when working with wide peaks, the chromatographic separation of MeHg and Hg(II) from other interfering Hg species is compromised and may lead to error in the measurement of species-specific isotopic compositions in real samples. Our results demonstrate for the first time that measuring Hg isotope ratios in transient signals of narrow GC peaks provides a better external precision than when working with wide chromatographic peaks allowing the possibility of processing a higher number of samples with a better chromatographic resolution and a higher sensitivity. Finally, we also evaluated the influence of the time-lag correction of the Faraday cups in the external precision of Hg species-specific delta values. **Figure S5** shows that time lag correction has no effect either in the accuracy or in the external precision of the delta values when applying either the LRS method or the PbP method.

3.4.2 Analysis of NIST-3133 and NIST RM 8610 versus NIST-3133 using the optimized conditions.

According to the results obtained in section 3.4.1 we selected the LRS method for data reduction, an integration time of 0.131 s and 321 acquisition points for further measurements. Using these conditions, we evaluated the accuracy and external precision of our method. First, we evaluated the external precision of the method by calculating the δ values and Δ values of NIST-3133 bracketed with NIST-3133 from the Hg(II)-specific isotope ratios measured in n=25 independent replicates measured in three different days. As can be observed in Table 2, the external precisions ranged from 0.24 to 0.49 ‰. Secondly, we calculated the δ values and Δ values of NIST RM 8610 (new UM-Almaden) bracketed with NIST-3133 from the Hg(II)-specific isotope ratios measured in n=25 independent replicates measured in three different days. As can be

observed in Table 2, the obtained δ values and Δ values, were in agreement with the certified values obtained by Cold Vapor Generation. The external precision of the δ values and Δ values obtained in the analysis of this material ranged from 0.21 to 0.40 ‰. The obtained external precision were similar to those previously reported[23] using wide chromatographic peaks and the LRS method (0.2-0.5‰ for δ^{202}Hg).

4. CONCLUSIONS

This is the first time that the accuracy and precision of Hg species-specific isotope ratios and delta values obtained by GC-MC-ICP-MS is evaluated with narrow GC peaks. Our work demonstrates that, in our instrument, a better external reproducibility is obtained when working with narrow peaks than when working with wider peaks obtained from the application of isothermal temperature programs. This could be attributed to the better sensitivity in the peak apex and the increased number of data points with short integration times provided when working with narrow peaks. However, in comparison with previously published approaches based on wide GC peaks[23] we provide the same level of accuracy and external reproducibility of Hg compound specific delta values. The measurement of isotope ratios in narrow transient signals provides two important advantages when analyzing real samples. First, the analysis time per sample is reduced as the use of isothermal GC temperature programs requires significantly longer chromatographic runs than those require in conventional GC separations. Secondly, a better chromatographic resolution is obtained. The chromatographic separation of MeHg and Hg(II) from other interfering Hg species may be compromised when analyzing real samples leading to errors in the measurement of species-specific isotopic compositions. A higher sample throughput and a lower risk of interferences is thus provided with the methodology developed in this work in comparison with previously reported approaches.

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TABLES

Table 1. GC-MC-ICPMS operating conditions.

GC	**Agilent 6890N**		
Carrier gas	He		
Column	DB-5MS (5% diphenyl, 95% dimethylsiloxane, 30 m×0.53 mm i.d.×1.0 μm)		
Injector temperature	250 ºC		
Volume of injection	2 μL		
Injector purge time	1 min		
GC-ICP-MS Interface temperature	270 ºC		
GC program name	Program 1	Program 2	Program 3
Initial temperature	60ºC	45ºC	45ºC
Initial Time	1 min	7 min	1 min
Ramp 1	40ºC/min	50ºC/min	50ºC/min
Final temperature 1	250ºC	63ºC	74ºC
Final time 1	1 min	7 min	7 min
Ramp 2		50ºC/min	50ºC/min
Final temperature 2		250ºC	250ºC
Final time 2		1 min	1 min
Total analysis time	6.8 min	19.1 min	13.1 min

MC-ICP-MS	**Thermo Scientific - Neptune Plus**								
RF Power	1200 W								
Resolution mode	Medium								
Integration times	0.131 s or 0.262 s (program 1)				0.524 s or 1.049 s (program 2 or 3)				
Cycles/Blocks	2500 or 1200 (program 1)				1200 or 600 (program 2 or 3)				
Sample Ar gas flow	0.7 L/min								
Nebulization Ar gas flow	0.5 L/min								
Cooling Ar gas flow	15.2 L/min								
Auxiliary Ar gas flow	0.8 L/min								
Faraday Cups Configuration	L4	L3	L2	L1	C	H1	H2	H3	H4
		^{198}Hg	^{199}Hg	^{200}Hg	^{201}Hg	^{202}Hg	^{203}Tl	^{205}Tl	

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629 **Table 2.** Hg delta values of NIST SRM 3133 and NIST RM 8610 calculated versus
630 NIST SRM 3133. Uncertainty of the values corresponds to the external precision
631 expressed as 2SD of the mean of n=25 replicates measured in 3 different measurement
632 days for each material.

	$\delta^{199}Hg$	$\delta^{200}Hg$	$\delta^{201}Hg$	$\delta^{202}Hg$	$\Delta^{199}Hg$	$\Delta^{200}Hg$	$\Delta^{201}Hg$
NIST SRM 3133 (n=25)	0.00 ±0.29	0.02±0.43	0.01±0.49	0.05±0.43	-0.01±0.24	-0.01±0.29	-0.02±0.32
NIST RM 8610 (n=25)	-0.17±0.40	-0.28±0.38	-0.45±0.35	-0.60±0.34	-0.02±0.34	0.02±0.28	0.00±0.21
Certified values for NIST RM 8610	*-0.17±0.01*	*-0.27±0.02*	*-0.46±0.02*	*-0.56±0.03*	*-0.03±0.02*	*0.00±0.01*	*-0.04±0.01*

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FIGURES

Figure 1. Schematic analytical setup of the GC-MC-ICPMS used in this work for the measurement of compound-specific Hg isotope ratios.

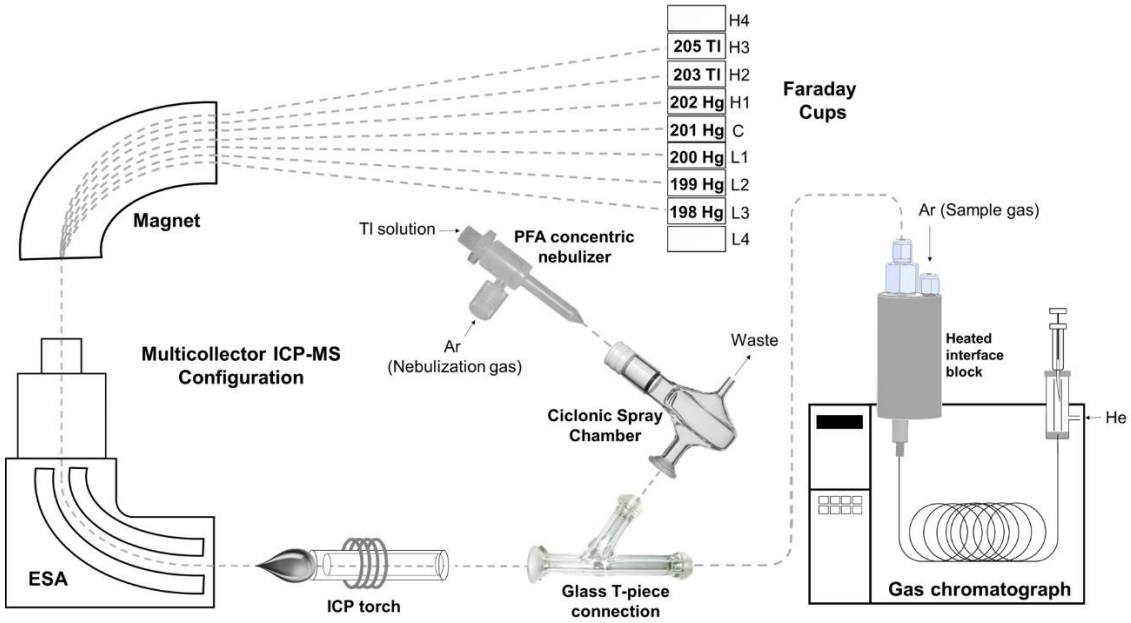

Figure 2. Optimization of Ar flows of the GC-MC-ICPMS hyphenated system used in this work.

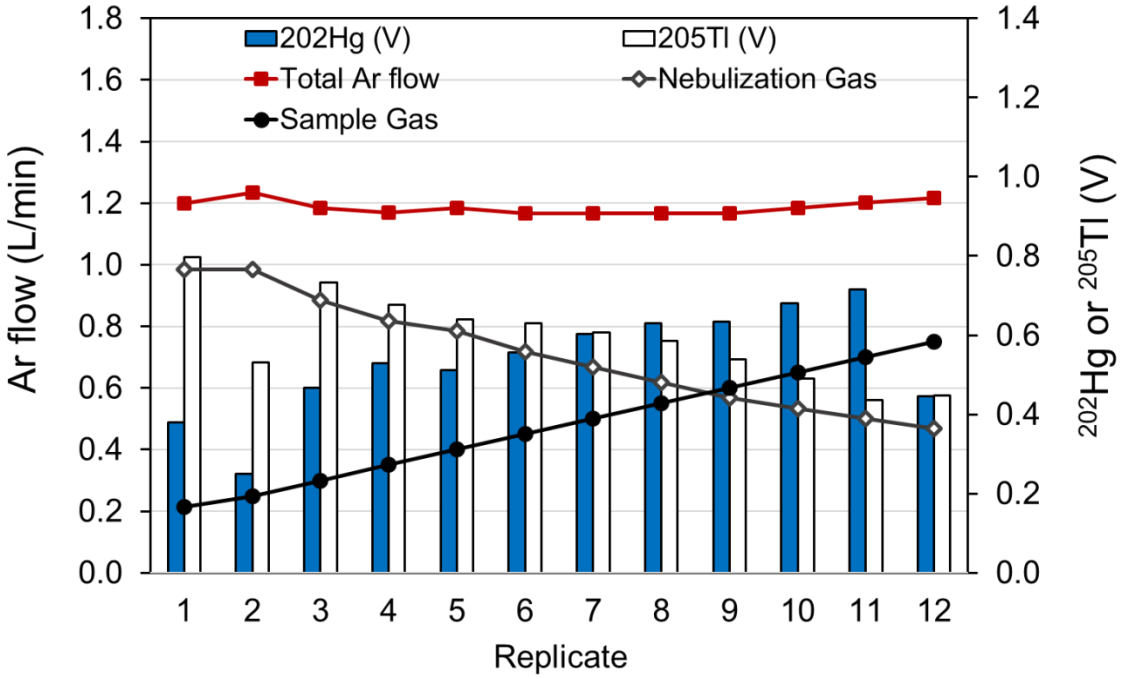

Figure 3. Comparison of the chromatographic peak profile of Hg(II) obtained for the isotope ^{202}Hg by GC-MC-ICP-MS injecting 2 µL of a solution containing 250 ng g^{-1} of Hg(II) when using a) T program 1, b) T program 2 and c) T program 3.

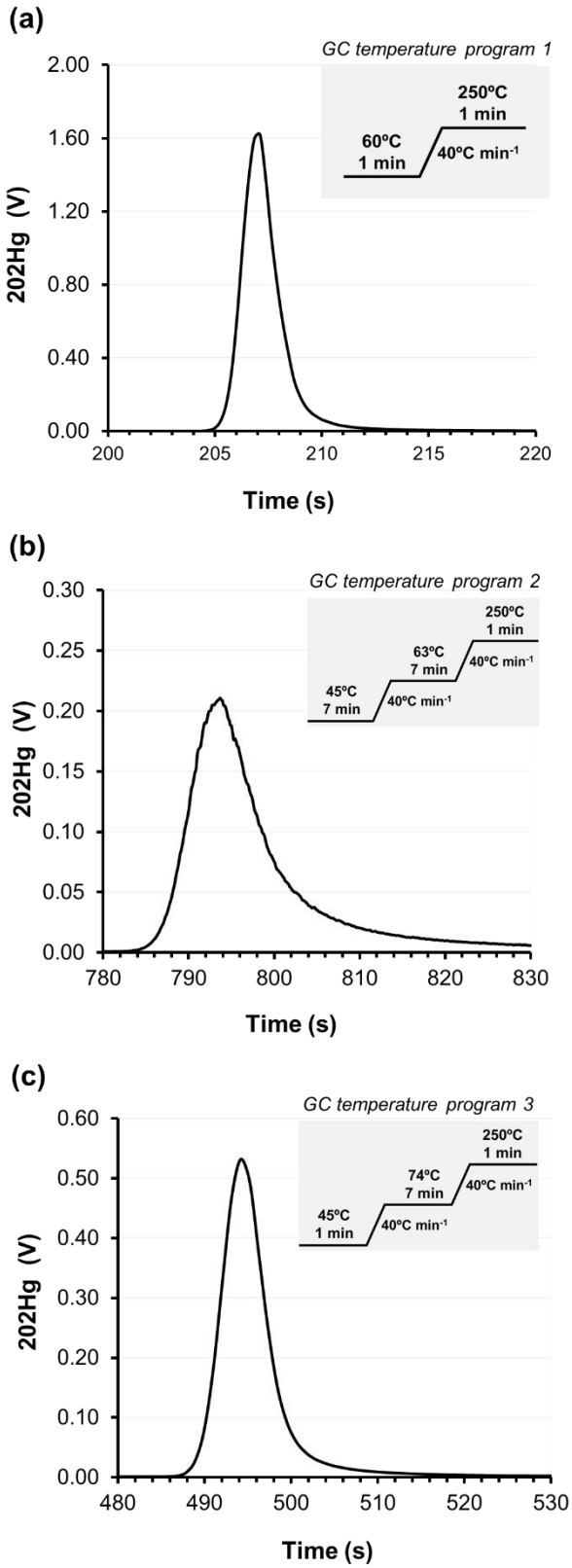

Figure 4. Example of a representative time-lag corrected and uncorrected $^{202}Hg/^{198}Hg$ isotope ratio (not corrected for mass bias) during Hg(II) elution when injecting 2 µL of a solution containing 250 ng g^{-1} of Hg(II) (NIST 3133) using integration times of a) 0.131 s and b) 0.262 s for narrow GC peaks, and c) 0.524 s and d) 1.049 s for wide GC peaks.

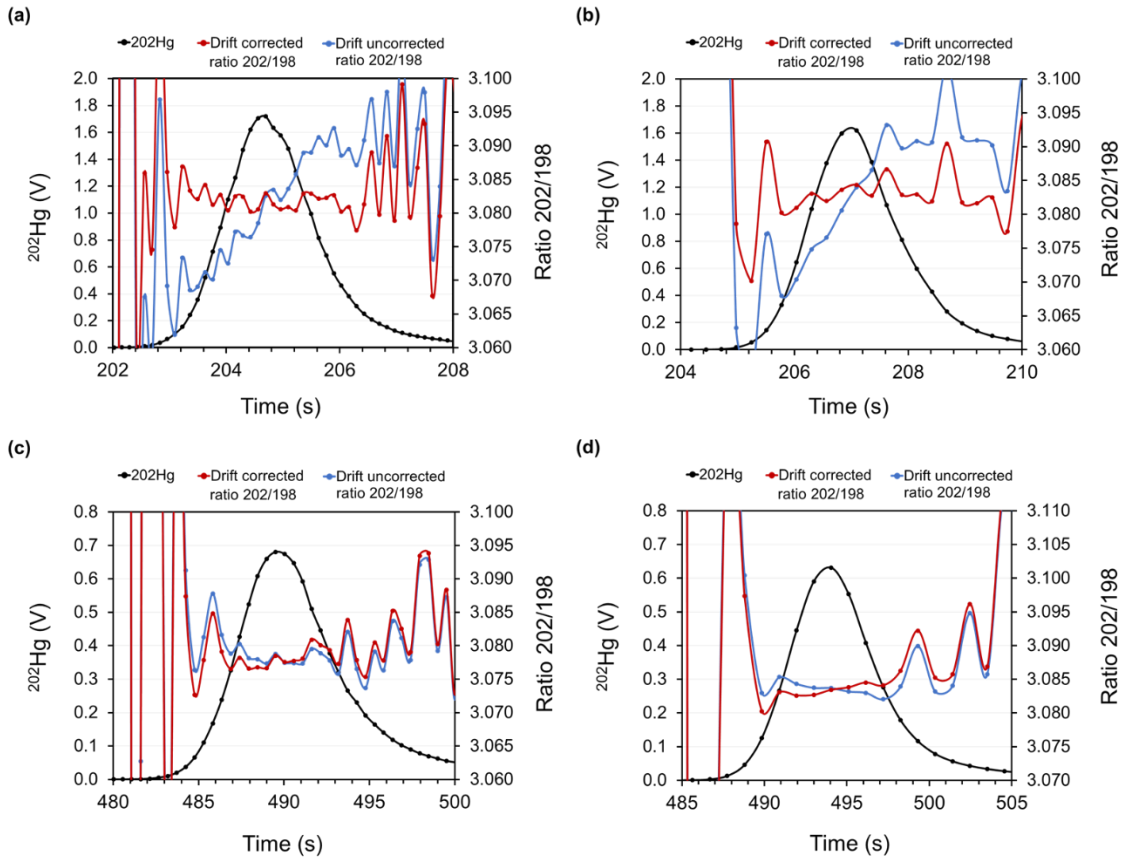

Figure 5. Time-lag corrected and uncorrected $^{202}Hg/^{198}Hg$ isotope ratios (mass bias corrected) of NIST 3133 measured by GC-MC-ICP-MS using: a) LRS method, narrow GC peak, 0.131 s integration time, 21, 81, 161 and 481 acquisition points; b) LRS method, wide GC peak, 1.049 s integration time, 21 and 161 acquisition points; c) PbP method, narrow GC peak, 0.131 s integration time and 5 acquisition points; d) PbP method, wide GC peak, 1.049 s integration time and 5 acquisition points. Error bars correspond to the internal precision of the isotope ratios expressed as two times the standard eror (2SE)

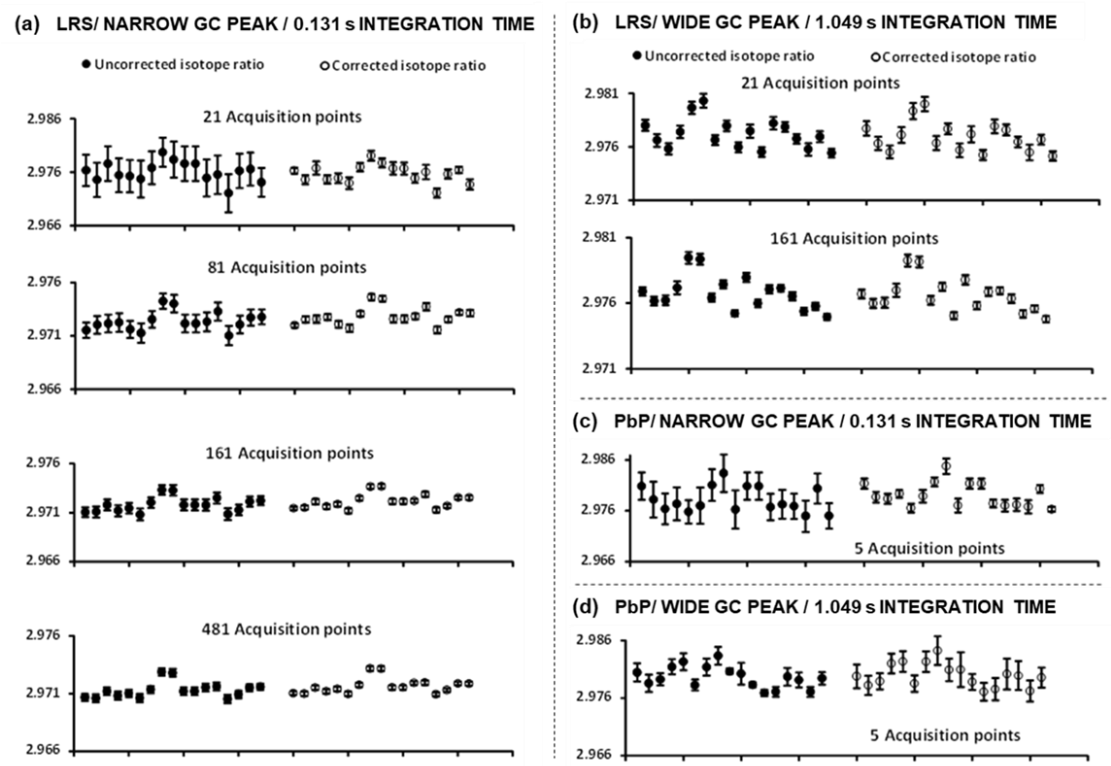

Figure 6. δ^{202}Hg(II) (‰) obtained under different experimental conditions when analyzing NIST-3133 versus NIST-3133. Error bars represent the associated external precision (±2SD) for n=8 independent measurements. An optimal external precision interval of ±0.50 ‰ based on previous studies [14,23] is highlighted.