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Yang, L.; Sturgeon, R.



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# Blank correction considerations for isotope dilution and reverse isotope dilution calibration: Determination of methylmercury in fish tissue

**Lu Yang* and Ralph E. Sturgeon**

*Institute for National Measurement Standards, National Research Council Canada, Ottawa, Ontario, Canada K1A 0R6. E-mail: Lu.Yang@nrc.ca*

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A mathematical approach to the accurate correction of the blank when applying isotope dilution (ID) and reverse ID is presented. The manner in which blank correction is undertaken is critical to the quality of the final results. Direct subtraction of a procedural blank from the gross analyte concentration is only valid when the blank contributes to the primary ID process and not to the reverse ID process. When the blank contributes to both processes, typically only a fraction of this blank concentration should be subtracted. The approach developed here was illustrated and validated by the determination of MeHg in tuna fish using ID and reverse ID SPME GC-ICP-MS and an enriched $Me^{198}Hg$ spike. Despite a 150-fold higher blank (equivalent to 7% of the analyte concentration in the sample) arising from use of a 1 M NaOAc/HOAc buffer solution compared to that obtained with use of a 0.5 M $NH_4OAc$/HOAc buffer, final concentrations of 19.90 ± 0.34 and 19.88 ± 0.10 μmol $kg^{-1}$ (one standard deviation, $n = 3$) respectively, were derived. These data are in good agreement with the assigned value of 19.91 ± 0.82 μmol $kg^{-1}$ (as 95% confidence interval, derived from an international intercomparison exercise). The methodology was also applied to the determination of a 50-fold lower concentration of MeHg in a salmon fish. A method detection limit (3SD) of 0.9 nmol $kg^{-1}$ based on processing of a 0.40 g subsample was obtained.

## Introduction

The application of isotope dilution (ID) inductively coupled plasma mass spectrometry (ICP-MS) for total trace metal analysis and trace metal speciation has increased significantly over the last decade. ID generally provides the ultimate approach to high accuracy and precision in results.[1] The ID technique is based on the addition of an enriched spike to a sample to obtain analyte concentration from the altered isotope ratio. The concentration of the spike is, in turn, usually determined by reverse ID against a natural abundance primary standard. Once isotopic equilibration is achieved between the endogenous analyte in the sample and the added spike, ID ICP-MS is capable of compensating for subsequent loss of some analyte during sample manipulation, suppression of ion sensitivities by concomitant elements present in the sample matrix and for instrument drift. ID ICP-MS is considered to be a primary method of analysis.[2]

Although ID methodology is capable of compensating for many analytical pitfalls, random contamination introduced from environmental sources occurring at stages after the sample and spike have equilibrated is not one of them. Thus, every effort must be made to minimize such occurrences. Proper ID methodology with blank correction is capable of accurately compensating for systematic contamination introduced through use of reagents. As the blank can never be completely eliminated, accurate blank correction is important, especially for ultratrace analysis and those samples demanding the utmost in accuracy. Common practice for analysis of total trace metal content using ID generally involves subtraction of a procedural blank from the gross computed analyte concentration to yield a net result. In such cases, the blank contributes to the ID procedure through various reagents used during sample preparation and is characterized by its natural isotopic abundance. By taking into consideration contribution from a blank, the following equation can be used to calculate the net analyte concentration in a sample using ID:[3]

$$C_x \cdot (w \cdot m_x) + b = C_y \cdot m_y \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \qquad (1)$$

where $C_x$ is the final analyte concentration (μmol $kg^{-1}$) in the sample based on dry mass; $C_y$ is analyte concentration (μmol $kg^{-1}$) in the enriched spike; $m_y$ is the mass (kg) of spike used to prepare the blend solution of sample and spike; $m_x$ is the mass (kg) of sample used; $w$ is the dry mass correction factor; $b$ is the magnitude of the blank (μmol); $A_y$ is the abundance of reference isotope in the spike; $B_y$ is the abundance of spike isotope in the spike; $A_x$ is the abundance of reference isotope in the sample; $B_x$ is the abundance of spike isotope in the sample and $R_n$ is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike. Eqn. (1) can also be expressed in the following form:

$$C_x = \frac{C_y \cdot m_y}{w \cdot m_x} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} - \frac{b}{w \cdot m_x} = \frac{C_y \cdot m_y}{w \cdot m_x} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} - C_b \qquad (2)$$

where $C_b$ is the blank concentration normalized to sample weight (μmol $kg^{-1}$). For most trace inorganic analyses, no blank correction is needed in the reverse ID process to obtain an accurate measure of analyte in the enriched spike since minimal amounts of reagents that ideally contribute negligibly to the blank are usually used. Therefore, the following equation can be used to calculate the analyte concentration in a spike using reverse ID under this condition:

$$C_y = \frac{C_z \cdot m_z}{m_y'} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} \qquad (3)$$

where $m_y'$ is the mass (kg) of spike used to prepare the blend solution of spike and natural abundance primary standard

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solution; $C_z$ is the concentration of natural abundance primary standard (μmol kg$^{-1}$); $m_z$ is the mass (kg) of natural abundance primary standard used; $A_z$ is the abundance of reference isotope in the natural abundance primary standard; $B_z$ is the abundance of spike isotope in the natural abundance primary standard and $R_n'$ is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and natural abundance analyte standard. Substituting eqn. (3) into eqn. (2), a general eqn. (4) is derived:

$$C_x = C_z \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} - C_b \quad (4)$$

This equation can be used for calculation of the net analyte concentration when using both ID and reverse ID techniques, wherein correction for the blank is accurately accounted for as it contributes only to the primary ID process and not the reverse ID procedure.

More recently, a number of species specific isotopically enriched spikes have become available to permit application of ID for trace metal speciation analysis. Since most such spikes are frequently synthesized in the users' laboratory, both the purity and stability of the enriched spikes is often a concern. Reverse ID for accurate quantitation of the concentration of an enriched spike against a natural abundance high-purity standard is thus normally undertaken to establish an accurate spike concentration. As derivatization of analytes to more volatile compounds is typically required when GC methods are used for separation and sample introduction, the blank contributed from reagents used in such procedures manifests itself in the reverse ID process as well and can no longer be conveniently neglected. Thus, eqn. (4) is no longer valid and a suitable equation is needed to achieve correction for the blank in this situation.

As noted above, blanks arising from various reagents have a natural isotopic abundance signature for the analyte. This blank thus contributes to the natural abundance primary standard concentration in the reverse ID process. In such a case, the following equation can be used to calculate the analyte concentration in a spike using reverse ID:

$$C_y = \frac{C_z \cdot m_z + b}{m_y'} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} \quad (5)$$

where $m_y'$ is the mass (kg) of spike used to prepare the blend solution of spike and natural abundance primary standard solution; $C_z$ is the concentration of natural abundance primary standard (μmol kg$^{-1}$); $m_z$ is the mass (kg) of natural abundance primary standard used; $A_z$ is the abundance of reference isotope in the natural abundance primary standard; $B_z$ is the abundance of spike isotope in the natural abundance primary standard and $R_n'$ is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and natural abundance analyte standard. Eqn. (5) can also be expressed in the following form:

$$C_y = \frac{C_z \cdot m_z}{m_y'} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} + \frac{b}{m_y'}$$

$$= \frac{C_z \cdot m_z}{m_y'} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} + C_b^{\mathrm{RSID}} \quad (6)$$

where $C_b^{\mathrm{RSID}}$ is the blank concentration normalized to spike weight (μmol kg$^{-1}$). Clearly, unlike blank correction in a simple ID process, wherein it is subtracted from the computed gross analyte concentration in the sample (evident in eqn. (2)), blank correction for a reverse ID process requires addition of this blank contribution to the computed gross analyte concentration in a spike to achieve the final accurate concentration of an enriched spike, as evident in eqn. (6).

Unfortunately, such details of implementation of blank correction with ID and reverse ID techniques are rarely discussed in the literature. The objective of this study was to develop an equation which permits a simplified approach to the implementation of the full ID process to permit accurate blank correction for species specific determinations when ID and reverse ID are utilized. This mathematical approach was validated by the determination of MeHg in a tuna fish sample used earlier in an international intercomparison exercise organized by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) of the European Commission for the Comité Consultatif pour la Quantité de Matière (Consultative Committee for Amount of Substance, CCQM) in August 2004. The methodology was also applied to the determination of a 50-fold lower concentration of MeHg in a salmon fish as part of a subsequent CCQM key comparison (K-43) exercise.

## Experimental

### Instrumentation

A ThermoFinnigan Element2 sector field ICP-MS instrument (Bremen, Germany) equipped with a Scott-type double pass glass spray chamber and a PFA self aspirating nebulizer was used. Optimization of the Element2 was performed as recommended by the manufacturer. Detector dead time was determined following the procedure of Nelms *et al.*[4] (method 2) whereby a plot of $^{238}U/^{235}U$ ratio *versus* U concentration was constructed using solution concentrations of 0.5, 1.0 and 2.5 ng ml$^{-1}$, from which a dead time of 18 ns was derived.

A Varian 3400 gas chromatograph (Varian Canada Inc. Georgetown, Ontario, Canada) equipped with a MXT-5 metal column (5% diphenyl, 95% polydimethylsiloxane, 30 m × 0.28 mm i.d. with a 0.5 μm film thickness) was used for separation of the mercury species. The GC was coupled to the ICP torch using a home-made interface and transfer line, as described in detail previously.[5,6] Typical operating conditions for the GC-ICP-MS system are summarized in Table 1.

A manual SPME device, equipped with a fused silica fiber coated with a 100 μm film of polydimethylsiloxane (Supelco, Bellefonte, USA), was used for the sampling of propylated MeHg from the headspace above its aqueous solutions. For convenience, SPME sampling was conducted in a regular fumehood.

### Reagents and solutions

A methanolic solution of potassium hydroxide (25% m/v) was prepared by dissolving KOH (Fisher Scientific, Nepean, Canada) in methanol. Acetic and hydrochloric acids were purified in-house prior to use by sub-boiling distillation of reagent grade feedstock in a quartz still. OmniSolv® methanol (glass-distilled) was purchased from EM Science (Gibbstown, NJ, USA). High purity de-ionized water (DIW) was obtained from a NanoPure mixed bed ion exchange system fed with reverse osmosis domestic feed water (Barnstead/Thermolyne Corp, Iowa, USA). A 5% (*m*/*v*) solution of sodium tetrapropylborate was prepared by dissolving $NaBPr_4$ (GALAB, Geesthacht, Germany) in DIW. A 0.5 M ammonium acetate buffer and a 1 M sodium acetate buffer (NaOAc) at pH 5 were prepared by dissolving appropriate amounts of $NH_4OAc$ (Fisher Scientific, Nepean, Ontario, Canada) and sodium acetate (EM Science, Darmstadt, Germany), respectively, in water and adjusting the pH with glacial acetic acid.

High purity methylmercury chloride was purchased from Sigma Aldrich Canada Ltd. (Oakville, ON, Canada). Individual stock solutions of 13 500–21 000 μmol kg$^{-1}$ were prepared in methanol and kept refrigerated until use. Natural abundance MeHg working standard solutions of 1.6511, 1.6922, 8.1433

**Table 1** GC and ICP-MS operating conditions

| | GC |
|---|---|
| Injection mode | Splitless |
| Injector temperature | 220 °C |
| Column | MXT-5 (30 m × 0.28 mm × 0.5 μm) |
| Carrier gas | He at 44 psi |
| Oven program | 50 °C (1 min) to 250 °C at 30 °C $min^{-1}$ (1 min) |
| Interface temperature | 250 °C |

| | ICP-MS |
|---|---|
| Rf power | 1200 W |
| Plasma Ar gas flow rate | 15.0 l $min^{-1}$ |
| Auxiliary Ar gas flow rate | 1.0 l $min^{-1}$ |
| Nebulizer Ar gas flow rate | 0.275 l $min^{-1}$ |
| Sampler cone orifice (nickel) | 1.00 mm |
| Skimmer cone orifice (nickel) | 0.88 mm |
| Lens voltage | Focus: −830 V; × deflection: −0.95 V; y deflection: 1.60 V; Shape: 105 V |
| Dead time | 18 ns |
| Resolution | 300 |
| Data acquisition | *E*-scan, 2500 passes, 5% mass window, 0.001 s settling time, 0.0050 s sample time. |

and 8.2733 μmol $kg^{-1}$ were prepared by diluting the stock solutions with 50% methanol in DIW.

$^{198}$Hg enriched MeHg spike solutions at nominal concentrations of 0.38 and 3.45 μmol $kg^{-1}$ in 50% methanol were prepared from an isotopically enriched $CH_3{}^{198}HgCl$ ($Me^{198}Hg$) stock synthesized in our laboratory from a commercially available inorganic $^{198}$Hg (96% isotopic purity).[7]

CCQM-K43 salmon fish and CCQM-P39 tuna fish were obtained from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium).

### Sample preparation and analysis procedure

Sample preparation was based on the procedure reported by Cai and Bayona.[8] Nominal 0.40 g subsamples spiked with an appropriate mass of enriched $Me^{198}Hg$ and 20 ml of methanolic KOH solution were mechanically shaken overnight and then stored at 4 °C until analyzed. Three replicate blanks (spiked with 10% of the amount of enriched isotope solution used for the samples) were processed along with the samples. Four reverse spike isotope dilution calibration samples were prepared to quantify the concentration of the enriched $Me^{198}Hg$ spike by mixing an appropriate mass of spike and natural abundance MeHg standard in a 20 ml methanolic KOH solution. For SPME headspace sampling, a 4.0 ml volume of digest or reverse spike isotope dilution calibration sample was transferred to a 22 ml glass vial. After 10 ml of 1 M NaOAc buffer solution (or 0.5 M $NH_4OAc$ buffer), 1.75 ml of HCl and 1 ml of 5% $NaBPr_4$ were added, the vial was capped with a PTFE coated silicone rubber septum. The SPME needle was inserted through the septum and headspace sampling was performed for 10 min under vigorous stirring. The collected analyte was then desorbed from the SPME fiber onto the GC column.

Following injection of the sample onto the GC column, data acquisition on the Element2 was manually triggered. Isotopes of $^{202}$Hg, $^{200}$Hg and $^{198}$Hg were monitored during every run. One ml of a 0.4 μmol $kg^{-1}$ natural abundance MeHg standard (candidate CRM material fully characterized for isotopic composition by National Research Council Canada and collaborating laboratories) was used for mass bias correction. At the end of the chromatographic run, the acquired data were transferred to an off-line computer used to calculate peak areas of the MeHg transients and to generate $^{200}Hg/^{198}Hg$ or $^{202}Hg/^{198}Hg$ ratios, from which the analyte concentrations in the fish tissues were calculated.

## Results and discussion

### Optimization of GC-ICP-MS and SPME sampling

Optimization of the ICP-MS system was undertaken as recommended by the manufacturer. Mass calibration was only performed once per week because of the good stability of the Element2. The plasma was then extinguished and the spray chamber and nebulizer assembly replaced with the GC transfer line and its ball joint adapter. Previous work[6] has shown that there is no significant difference in lens voltages, torch position and rf power for optimized wet and dry plasma conditions. Thus, only the few parameters noted below required optimization for dry plasma conditions, achieved by monitoring the response following injection of 1 μl of a 0.1 μmol $kg^{-1}$ propylated MeHg standard in iso-octane.

The distance between the injector tip and the end of the transfer line had no significant effect on the resulting sensitivity over the range 0 to 12 mm; consequently, a 5 mm distance was used in this study. It was also found that the length of the transfer line had little effect on the analyte peak shape, due to a short residence time. Therefore, for flexibility and ease of handling of the tandem GC-ICP-MS set-up, a 150 cm long PTFE line was used.

The Ar carrier gas flow rate is inter-related to that of the He effluent from the GC column as it was introduced through a side arm of the interface and was optimized for a He pressure of 44 psi. Ar carrier gas flow was optimized in the range of 0.2 to 0.45 l $min^{-1}$ by examining the response following injections of 1 μl of a 0.1 μmol $kg^{-1}$ propylated MeHg standard in iso-octane. Highest sensitivity was found using flow rates in the range of 0.250 to 0.280 l $min^{-1}$; sensitivity decreased gradually beyond this range and thus an optimum Ar carrier gas flow rate of 0.27 l $min^{-1}$ was selected for subsequent studies.

Headspace sampling was chosen to minimize exposure of the SPME fiber to the sample matrix, thereby enhancing the lifetime of the fiber. As SPME extraction efficiency can be influenced by a number of factors, including extraction temperature, extraction time, pH of the solution and the concentration of derivatization reagent, optimized parameters were taken from an earlier SPME GC-ICP-MS study.[9] Optimum sample pH was found to be in the range 4 to 7. A pH 5 obtained with a 0.5 M $NH_4OAc$ or 1 M NaOAc buffer was used. A severe matrix effect was encountered with the fish extracts, necessitating that a concentration of 5% $NaBPr_4$ be used to ensure maximum propylation of analyte in the sample matrix.

### Blank correction in isotope dilution and reverse isotope dilution calibration

In preliminary work, a high MeHg blank was obtained with use of a 1 M NaOAc buffer solution prepared from reagent grade NaOAc. Although the blank value decreased about 150-fold when a 0.5 M $NH_4OAc$ buffer solution was used, it still constituted several percent of the analyte concentration for the much lower MeHg encountered with the CCQM-K43 salmon fish. In order to maintain a constant blank in both ID and reverse ID processes, sample preparation was identical for both and analyses were conducted on the same day.

To obtain a uniform equation for accurate blank correction when using both ID and reverse ID techniques, eqn. (1) was divided by eqn. (5) and solved for $C_x$, yielding:

$$C_x = C_z \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} - \frac{b}{w \cdot m_x} \cdot \left(1 - \frac{m_y}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'}\right) \quad (7)$$

wherein the term $\frac{b}{w \cdot m_x}$ equals $C_b$ (μmol kg$^{-1}$), which is the sample blank normalized to dry sample weight. Substituting $f_b$ for $\left(1 - \frac{m_y}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'}\right)$, eqn. (8) arises:

$$C_x = C_z \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} - f_b \cdot C_b \quad (8)$$

This measurement equation can be simplified to eqn. (9) for all elements whose isotopic abundance is invariant in nature (and therefore $A_x = A_z = A_{xz}$, $B_x = B_z = B_{xz}$).

$$C_x = C_z \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R_n' - A_{xz}}{A_y - B_y \cdot R_n'} - f_b \cdot C_b \quad (9)$$

It is evident from eqn. (9) that only a fraction of the sample blank value is subtracted from the gross analyte concentration expressed by $C_x^{\text{gross}} = C_z \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R_n' - A_{xz}}{A_y - B_y \cdot R_n'}$, (the first term in eqn. (9)) if this blank contributes to both the primary ID and reverse ID processes. Eqn. (9) is significantly different from eqn. (4) wherein a full procedural blank is subtracted from the gross analyte concentration when the blank only contributes to the primary ID process and not to the reverse ID process.

If experiments can be designed to fulfil the following requirements: ID samples and reverse ID samples are prepared in the same way; the amount of spike used for ID ($m_y$) equals the amount ($m_y'$) used for reverse ID and the ratio achieved in the spiked sample equals the ratio in the reverse ID calibration blend, then the blank correction factor $f_b$ becomes zero. The blank contributed by the primary ID process cancels out the blank contributed by the reverse ID process. Therefore, eqn. (9) can be simplified to eqn. (10) for calculation of the final analyte concentration in a solid sample:

$$C_x = C_z \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R_n' - A_{xz}}{A_y - B_y \cdot R_n'} \quad (10)$$

In practice, it is virtually impossible to achieve these ideal conditions unless one uses a consecutive exact-matching ID approach.[10] This equation was simply developed to illustrate the mathematical feasibility of correcting for this inevitable blank, which contributes to both ID and reverse ID processes. Eqn. (10) is not recommended for measurements requiring the utmost accuracy.

### Validation of calibration approach for the determination of MeHg

Analysis of CCQM-P39 tuna fish with an assigned value for MeHg content was used to validate the proposed method. No significant difference in measured MeHg concentrations was obtained when either isotope pair $^{202}$Hg/$^{198}$Hg or $^{200}$Hg/$^{198}$Hg was used for the calculations based on eqn. (9). The $^{202}$Hg/$^{198}$Hg isotope pair was thus selected for the final quantitation of MeHg because of the higher abundance of this isotope (better sensitivity). As equilibration between the added spike and the endogenous analyte in the sample is a prerequisite for achieving accurate results using ID, spiked samples were digested with 25% methanolic KOH and stored in the dark at 4 °C for at least two days prior to ratio measurements. To verify equilibration, $^{202}$Hg/$^{198}$Hg and $^{200}$Hg/$^{198}$Hg ratios were re-measured two weeks later using one spiked sample which had been stored in the dark at 4 °C following the initial measurement. No significant difference was found between the two sets of results.

**Table 2** Results for MeHg amount content in CCQM-P39 tuna fish[a]

| Parameters | Buffer solutions: 1 M NaOAc/HOAc | Buffer solutions: 0.5 M $NH_4$OAc/HOAc |
|---|---|---|
| $C_x^{\text{gross}}$/μmol kg$^{-1}$ | 19.84 ± 0.29 | 19.88 ± 0.09 |
| $f_b$ | −0.043 ± 0.033 | −0.021 ± 0.006 |
| $C_b$/μmol kg$^{-1}$ | 1.394 ± 0.046 | 0.0095 ± 0.0003 |
| $C_x$/μmol kg$^{-1}$ | 19.90 ± 0.34 | 19.88 ± 0.10 |
| Assigned value/μmol kg$^{-1}$[b] | 19.91 ± 0.82 | 19.87 ± 0.09 |
| $C_x^{\text{gross}} - C_b$/μmol kg$^{-1}$ | 18.45 ± 0.29 | 19.87 ± 0.09 |

[a] Values are reported in mean and one standard deviation, $n = 3$. [b] With 95% confidence interval.

To validate use of eqn. (9), two different buffer solutions containing different contaminant concentrations of MeHg were used. Results are summarized in Table 2. Despite an approximately 150-fold higher blank value (1.394 ± 0.046 μmol kg$^{-1}$, one standard deviation, $n = 3$) obtained with use of the 1 M NaOAc/HOAc buffer solution (compared to a concentration of 0.0095 ± 0.0003 μmol kg$^{-1}$, one standard deviation, $n = 3$ for the 0.5 M $NH_4$OAc/HOAc buffer), a final concentration of MeHg of 19.90 ± 0.34 μmol kg$^{-1}$ ($C_x$, one standard deviation, $n = 3$) calculated with use of eqn. (9) and the 1 M NaOAc/HOAc buffer solution is in good agreement with the 19.88 ± 0.10 μmol kg$^{-1}$ concentration obtained using the 0.5 M $NH_4$OAc/HOAc buffer solution. These results are also in good agreement with the CCQM-P39 assigned value of 19.91 ± 0.82 μmol kg$^{-1}$ (uncertainty reported as 95% confidence interval).[11] Simple use of eqn. (4) for the blank correction in this situation generates an inaccurate correction when using the 1 M NaOAc/HOAc buffer solution ($C_x^{\text{gross}} - C_b$ = 18.45 ± 0.29 μmol kg$^{-1}$) because the blank contributes to both ID and reverse ID processes (Table 2).

It is interesting to note that $f_b$ values obtained with two different buffer solutions are indeed quite small because sample preparation for ID and reverse ID was performed in such a manner as to nearly satisfy the requirements delineated for eqn. (10). Thus, blank contributions to ID and reverse ID processes cancelled out. As evident in Table 2, no significant difference in MeHg concentrations ($C_x^{\text{gross}}$) is obtained using eqn. (10) with either buffer solution. As noted earlier, this equation was simply developed for mathematical convenience for correction of a blank which contributes to both ID and reverse ID processes. It is not recommended for measurements requiring the utmost accuracy since it is virtually impossible to fully satisfy the requirements of eqn. (10) unless one uses an exact-matching ID approach. For this study, eqn. (9) was thus used for the final quantitation of MeHg in CCQM-K43 salmon fish.

### Application of calibration approach to the determination of MeHg in CCPM-K43 Salmon

As evident from the chromatograms in Fig. 1, the concentration of MeHg in CCQM-K43 salmon fish is much lower than that in CCQM-P39 tuna fish. Thus, contamination control during sample preparation and accurate blank correction are extremely critical to this sample. ID and reverse ID samples

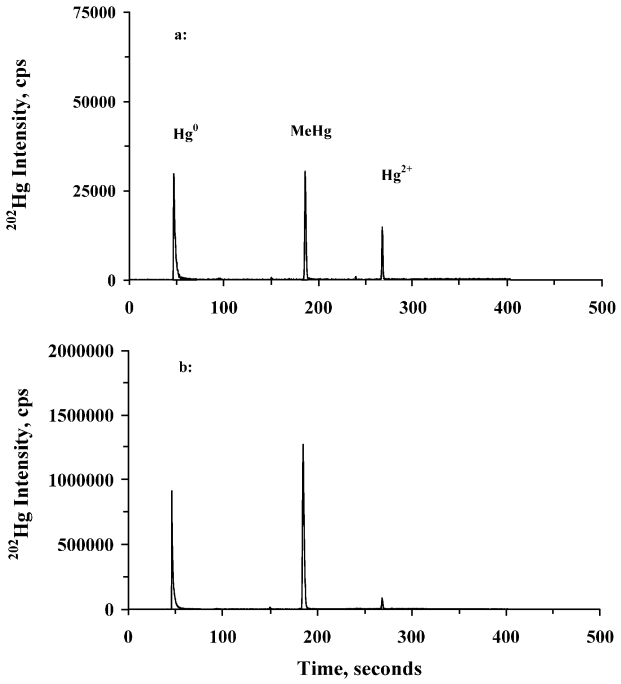


**Fig. 1** Chromatograms of fish extracts obtained by SPME-GC-ICP-MS; (a) CCQM-K43 salmon fish; (b) CCQM-P39 tuna fish.

were prepared in the same way with use of the same amount of enriched $Me^{198}Hg$ spike and reagents. These samples were run on the same day following the SPME GC-ICP-MS protocol. The 0.5 M $NH_4OAc$/HOAc buffer solution was used for sample preparation because of its lower contribution to the blank. The concentration of MeHg was calculated using eqn. (9) based on the $^{202}Hg/^{198}Hg$ isotope pair. A MeHg concentration of $0.375 \pm 0.019$ μmol $kg^{-1}$ (total combined uncertainty, $k = 2$) was obtained for the CCQM-K43 salmon fish, 50-fold lower than that in the CCQM-P39 tuna fish.

The detection limit for the combined procedure using both ID and reverse ID with SPME GC-ICP-MS detection was estimated from the response from measurements of three $Me^{198}Hg$ spiked blanks. A value of 0.9 nmol $kg^{-1}$ was obtained, based on three times the standard deviation of measured concentrations normalized to a 0.40 g subsample, clearly adequate for the determination of MeHg in this salmon fish.

### Estimated total combined standard uncertainty

According to ISO Guide to the Expression of Uncertainty in Measurement,[12] the combined standard uncertainty of a measurement result $y$, designated $u_c(y)$, can be obtained from the following equation:

$$u_c^2(y) = \sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i}\right)^2 u^2(x_i) + 2 \cdot \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \left(\frac{\partial f}{\partial x_i}\right) \cdot \left(\frac{\partial f}{\partial x_j}\right) \cdot u(x_i, x_j) \quad (11)$$

Where $y = f(x_1, x_2, \ldots, x_N)$. The partial derivatives $\partial f/\partial x_i$ are often referred to as *sensitivity coefficients*, $u(x_i)$ is the standard uncertainty associated with the input $x_i$ and $u(x_i, x_j)$ is the estimated covariance associated with $x_i$ and $x_j$. Eqn. (11) can also be expressed as:

$$u_c^2(y) = \sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i}\right)^2 u^2(x_i) + 2 \cdot \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \left(\frac{\partial f}{\partial x_i}\right) \cdot \left(\frac{\partial f}{\partial x_j}\right) \cdot u(x_i) \cdot u(x_j) \cdot r_{(i,j)} \quad (12)$$

where $r_{(i,j)}$ is a correlation coefficient, $-1 \leq r_{(i,j)} \leq 1$. Eqn. (12) was used for estimation of total combined uncertainty of MeHg amount content in CCQM-K43 salmon fish following ID and reverse ID SPME GC-ICP-MS analysis. Individual partial derivatives were derived for all variables in eqn. (9) ($f_b$ and $C_b$ are treated as independent parameters since the value of $f_b \cdot C_b$ is generally quite small compared to the analyte concentration). The various components contributing to the total combined standard uncertainty are summarized in Table 3. As expected, the combined standard uncertainty for MeHg amount content in CCQM-K43 salmon fish is much larger than the simple standard deviation calculated from replicate measurements, which can be quite small due to the high precision achieved. Uncertainties associated with preparation of the primary assay standard of a natural abundance MeHg solution and the measurement of the isotopic ratio in the

**Table 3** Components of uncertainty for measurement of MeHg in CCQM-K43 salmon fish

| Parameter | Typical value | Type A/B | $u(x_i)$ | $\left(\frac{\partial f}{\partial x_i}\right)$ | $\left(\frac{\partial f}{\partial x_i}\right) \cdot u(x_i)$ |
|---|---|---|---|---|---|
| $C_z$/μmol $kg^{-1}$ | 1.6922 | B | 0.00527 | 0.2198 | 0.001 159 |
| $m_y$/kg | 0.000 4947 | B | 0.000 000 191 | 751.8035 | 0.000 144 |
| $m_x$/kg | 0.000 4209 | B | 0.000 000 191 | −883.6237 | −0.000 169 |
| $w$ | 0.97755 | A | 0.002 252 | −0.3805 | −0.000 857 |
| $m_z$/kg | 0.000 5211 | B | 0.000 000 191 | 713.7156 | 0.000 136 |
| $m_y'$/kg | 0.000 5057 | B | 0.000 000 191 | −735.4503 | −0.000 140 |
| $A_y$ | 0.0786 | B | 0.0019630 | −0.0127 | −0.000 025 |
| $B_y$ | 96.351 | B | 0.023498 | 0.000 010 | 0.000 000 243 |
| $A_{xz}$ | 29.8630 | B | 0.0330000 | 0.0048 | 0.000 159 646 |
| $B_{xz}$ | 9.9680 | B | 0.0130000 | −0.0145 | −0.000 188 |
| $R_n$ | 0.232 26 | B | 0.005146 | 1.7415 | 0.008 961 |
| $R_n'$ | 0.961 32 | B | 0.005 03 | −0.5700 | −0.002 868 |
| $f_b$ | −0.852 04 | A | 0.002 87 | −0.0082 | −0.000 024 |
| $C_b$/μmol $kg^{-1}$ | 0.0082 | A | 0.000 49 | 0.8520 | 0.000 414 |
| $2 \cdot \left(\frac{\partial f}{\partial A_y}\right) \cdot \left(\frac{\partial f}{\partial B_y}\right) \cdot u(A_y) \cdot u(B_y) \cdot r_{(A_y,B_y)}$, $r_{(A_y,B_y)} = -1$ used to obtain maximum value. | | | | | 0.000 000 000 012 |
| $2 \cdot \left(\frac{\partial f}{\partial A_x}\right) \cdot \left(\frac{\partial f}{\partial B_x}\right) \cdot u(A_x) \cdot u(B_x) \cdot r_{(A_x,B_x)}$, $r_{(A_x,B_x)} = -1$ used to obtain maximum value. | | | | | 0.000 000 0602 |
| Combined uncertainty/μmol $kg^{-1}$, $u_c$. | | | | | 0.00954 |

spiked sample and in the reverse ID calibration solutions are the principal sources contributing to the total combined standard uncertainty.

## Conclusion

A simplified approach for accurate correction of the blank during the determination of MeHg content in fish samples using both species specific ID and reverse ID with SPME GC-ICP-MS has been presented. The manner in which the blank correction is undertaken is critical to the quality of the final results. When a blank contributes to both ID and reverse ID processes, only a fraction of this blank concentration is subtracted from the gross analyte concentration (eqn. (9)). When a blank contributes only to the ID process and not to the reverse ID process, the full blank must be subtracted from the gross analyte concentration (eqn. (4)). As demonstrated in this study, accurate concentrations of MeHg in CCQM-P39 tuna fish were achieved despite use of a relatively contaminated NaOAc/HOAc buffer solution (contributing about 7% of the total MeHg concentration in the sample). The methodology outlined here is not only well suited for the accurate determination of MeHg in fish, but for trace analysis in general when a significant blank is encountered in both ID and reverse ID processes.

## References


1 P. De Bièvre, in *Isotope Dilution Mass Spectrometry in Trace Element Analysis in Biological Specimens*, ed. R. F. M. Herber and M. Stoeppler, Elsevier, Amsterdam, 1994.
2 Report of the 5th meeting of the Comité Consultatif pour la Quantité de Matière, BIPM, Feb., 1999.
3 K. G. Heumann, in *Isotope Dilution Mass Spectrometry in Inorganic Mass Spectrometry*, ed. E. Adams, R. Gijbels and R. Van Grieken, John Wiley & Sons, Chichester, UK, 1988.
4 S. M. Nelms, C. R. Quétel, T. Prohaska, J. Vogl and P. D. P. Taylor, *J. Anal. At. Spectrom.*, 2001, **16**, 333–338.
5 L. Yang, Z. Mester and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2002, **17**, 944–949.
6 L. Yang, Z. Mester and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2003, **18**, 1365–1370.
7 C. Bancon-Montigny, L. Yang, V. Colombini, Z. Mester and R. E. Sturgeon, *Appl. Organomet. Chem.*, 2004, **18**, 57–64.
8 Y. Cai and J. M. Bayona, *J. Chromatogr., A*, 1995, **696**, 113–122.
9 L. Yang, Z. Mester and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2003, **18**, 431–436.
10 R. B. Myors, R. Hearn and L. G. Mackay, *J. Anal. At. Spectrom.*, 2005, **20**, 216–219.
11 Y. Aregbe, C. Quétel and P. D. P. Taylor, CCQM-P39 Report, Aug., European Commission, Institute for Reference Materials and Measurements, Geel, Belgium, 2004.
12 Guide to the Expression of Uncertainty in Measurement, International Organisation for Standardisation, ISO/GUM, Geneva, Switzerland, 1995, ISBN 92-67-10188-9.