NRC Publications Archive Archives des publications du CNRC

Determination of methylmercury in fish tissues by isotope dilution SPME-GC-ICP-MS

Yang, L.; Mester, Z.; Sturgeon, R.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. /
La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version
acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien
DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1039/b301299a>

Journal of Analytical Atomic Spectrometry, 18, 5, pp. 431-436, 2003

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=302aa75b-924b-441e-b107-39626c53935>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=302aa75b-924b-441e-b107-39626c539353>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the
first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la
première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez
pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

Determination of methylmercury in fish tissues by isotope dilution SPME-GC-ICP-MS†

Lu Yang,* Zoltán Mester and Ralph E. Sturgeon

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

www.rsc.org/jaas

Received 3rd February 2003, Accepted 5th March 2003

First published as an Advance Article on the web 8th April 2003

A method is described for the accurate and precise determination of monomethylmercury (MMHg) by species specific isotope dilution (ID) calibration using solid phase microextraction (SPME) in combination with gas chromatographic (GC) separation and inductively coupled plasma mass spectrometric (ICP-MS) detection. Samples were digested with methanolic potassium hydroxide, derivatized in aqueous solution with sodium tetrapropylborate and headspace sampled with a polydimethylsiloxane coated SPME fused silica fiber. The analyte was then directly transferred from the fiber to the head of the GC column for desorption by insertion of the fiber through the heated injection port. Reverse spike ID analysis was performed to determine the accurate concentration of an in-house synthesized ^{198}Hg -enriched monomethylmercury (MM^{198}Hg) spike [candidate Certified Reference Material (CRM) EOM-1] using two natural abundance MMHg standards. Concentrations of 0.719 ± 0.015 and $4.484 \pm 0.029 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$) as Hg were obtained for MMHg in NRCC CRMs DOLT-2 and DORM-2, respectively, using the present method. These are in good agreement with the certified values of 0.693 ± 0.053 and $4.47 \pm 0.32 \mu\text{g g}^{-1}$ (as 95% confidence interval). A MMHg concentration of $4.69 \pm 0.12 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$) as Hg in DORM-2 was subsequently determined by standard additions calibration using ethylmercury (EtHg) as an internal standard. A nearly 4-fold improvement in the precision of determination using ID was obtained, clearly demonstrating its superiority in providing more precise results compared to the method of standard additions. The method was applied to the determination of MMHg in a new biological CRM DOLT-3 and a concentration of $1.540 \pm 0.025 \mu\text{g g}^{-1}$ (one standard deviation, $n = 6$) as Hg was obtained. A method detection limit (3σ) of 2.1 ng g^{-1} was estimated for a 0.25 g of subsample, sufficiently low for the routine determination of MMHg in most biological samples.

Introduction

Mercury has been introduced into the environment through both geochemical and anthropogenic sources,¹ resulting in a well-known environmental pollutant that exists in three major forms, elemental Hg^0 , a common form in air, inorganic Hg^{2+} (normally associated with ligands such as sulfur and humic substances) and organic Hg, and in particular, monomethylmercury (MMHg). MMHg is the most toxic form of mercury present in the environment and its bioaccumulation in fish provides the major route of exposure for humans. As a result, many countries have set guidelines for fish consumption to safeguard human health and efforts have been devoted to the development of sensitive, accurate and rapid analytical methods for monitoring MMHg in biological and environmental samples during the last two decades.

Determination of methylmercury in solid samples generally involves several analytical steps, including extraction, derivatization (where GC separation is involved), separation and detection. The most frequently used procedures for the extraction of mercury species from solid samples are based on alkaline^{2–6} and acidic leaching,^{7,8} aqueous distillation,^{9–13} supercritical fluid extraction¹⁴ and microwave-assisted extraction.^{15–17} Grignard reagents,^{18–21} sodium tetraethylborate (NaBEt_4),^{17,22–26} sodium tetrapropylborate (NaBPr_4)^{18,27} and sodium tetraphenylborate (NaBPh_4)^{28–33} have been used for efficient derivatization. Gas chromatography, capillary electrophoresis or liquid chromatography are commonly

used separation techniques for MMHg speciation in combination with atomic fluorescence spectrometry (AFS),^{3,34–36} electron-capture detection (GC-ECD),³⁷ atomic absorption spectrometry (AAS),^{16,22} ion trap mass spectrometry,^{30,31} inductively coupled plasma and microwave-induced plasma atomic emission spectrometry (ICP-AES, MIP-AES)^{14,21,38–41} and inductively coupled plasma mass spectrometry (ICP-MS) detection.^{18,24,36,40,42,43}

Over the past decade, the coupling of GC to ICP-MS has become a particularly popular technique for speciation work due to the high temporal resolution offered by GC and the high sensitivity, large dynamic range and multi-elemental capability of ICP-MS. On the other hand, sample preparation for GC analysis is usually time-consuming, and organic solvents used in the liquid–liquid extraction can be toxic. In an effort to simplify sample preparation, while retaining the merits of GC, solid phase microextraction (SPME) was introduced by Pawliszyn and co-workers^{44,45} in the early 1990s. Since 1993, following the commercialization of SPME, this sampling technique has gained widespread acceptance as a consequence of its simplicity, relatively low cost and ease with which analytes can be transferred to the GC column. The drawback noted with this technique is its low precision (typically 10% RSD). As the volume of the extraction phase provided by the fiber is extremely small, any irregularity/inhomogeneity in the polymer phase/surface may impart a significant effect on its extraction characteristics.^{46,47} In addition, because some level of degradation of the fiber generally occurs during repeated usage, the accuracy and precision achieved with the SPME technique can be compromised. The imprecision of the results obtained with different fibers often makes it necessary to

†Crown Copyright Canada 2003.

employ an internal standard or method of standard additions for calibration.⁴⁷ More recently, SPME has been applied to the determination of MMHg as an elegant, solvent-free sample extraction technique.^{6,27,33,41,48–50} The precision of results obtained using this technique is typically in the range of 2 to 10% RSD for MMHg.

In addition to the previously noted benefits of the ICP-MS detection technique, if two interference free isotopes of a given element are available the isotope dilution (ID) calibration strategy can be applied, which generally provides superior accuracy and precision over other calibration strategies, including external calibration and standard additions. This arises because a ratio, rather than an absolute intensity measurement, is used for quantitation of the analyte concentration.⁵¹ The ID-MS technique is considered to be a primary method of analysis because of its capability for high accuracy and precision.⁵² Applications of ID-ICP-MS to species specific determinations have been limited due to the need to synthesize species specific spikes. If these are available and isotopic equilibrium is achieved between the added spike and the analyte in the sample prior to ratio measurements, a number of advantages accrue, including: enhanced precision and accuracy in results as the species specific spike serves as an ideal internal standard; matrix effects are accounted for since quantitation is done by ratio measurements; non-quantitative analyte recovery does not impact on the final results; species alteration during sample work-up can be assessed;⁵³ and an alternative and comparative quantitation strategy is provided. However, despite the advantages offered by species specific isotope dilution methodology, few applications of ID for the determination of MMHg have been reported.^{23,24,54–60}

The objective of this study was to take advantage of the high accuracy and precision offered by ID in combination with the ease of use, simple and efficient sampling of SPME coupled with the superior separation and detection powers of GC-ICP-MS for MMHg determination in the certification of new CRM Dogfish Liver, DOLT-3. A reverse spike isotope dilution approach was performed to quantify the concentration of enriched MM¹⁹⁸Hg spike synthesized in house.⁶¹ The method was validated by the determination of MMHg in National Research Council of Canada CRMs DOLT-2 and DORM-2, which also served to demonstrate the high accuracy and enhanced precision offered by this methodology.

Experimental section

Instrumentation

The ICP-MS used in this work was a PerkinElmer SCIEX ELAN 6000 (Concord, Ontario, Canada) equipped with a Gem cross-flow nebuliser. A custom-made quartz sample injector tube (0.9 mm id) was used. A double-pass Rytan[®] spray chamber was mounted outside the torch box and maintained at room temperature. Optimization of the ELAN 6000 and dead time correction were performed as recommended by the manufacturer.

A Varian 3400 gas chromatograph (Varian Canada Inc. Georgetown, Ontario, Canada), equipped with an MXT-5 metal column (5% diphenyl, 95% polydimethylsiloxane, 20 m × 0.28 mm id with a 0.5 µm film thickness) was used for mercury species separations. The GC was coupled to the ICP-MS using a home-made interface and transfer line, as described in detail previously.⁶² 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 MMHg from the headspace above the aqueous solutions. For convenience, SPME sampling was conducted in a regular fume hood.

Table 1 GC and ICP-MS operating conditions

GC	
Injection mode	Splitless
Injection volume	1 µl isooctane
Injector temperature	220 °C
Column	MXT-5 (20 m × 0.28 mm × 0.5 µm)
Carrier gas	He at 26 psi
Oven program	50 °C (1 min) to 180 °C at 20 °C min ⁻¹ , to 250 °C at 30 °C min ⁻¹ (2 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	0.425 L min ⁻¹
Sampler cone orifice (nickel)	1.00 mm
Skimmer cone orifice (nickel)	0.88 mm
Lens voltage	8.75 V
Scanning mode	Peak hopping
Points per peak	1
Dwell time	40 ms
Sweeps per reading	1
Readings per replicate	5000
Number of replicates	1
Dead time	50 ns

Reagents and solutions

Potassium hydroxide methanolic solution, 25% (m/v), was prepared by dissolving KOH (Fisher Scientific, Nepean, Canada) in methanol. Acetic acid was 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., IA, USA). Sodium tetrapropylborate solution, 1% (m/v), was prepared by dissolving NaBPr₄ (GALAB, Geesthacht, Germany) in DIW. A 1 mol l⁻¹ sodium acetate buffer (NaAc) was prepared by dissolving an appropriate amount of sodium acetate (Fisher Scientific, Nepean, Ontario, Canada) in water and adjusting the pH to 5 with acetic acid.

Methylmercury chloride and ethylmercury chloride were purchased from Alfa Aesar (Ward Hill, MA, USA). Individual stock solutions of 1000–1500 µg ml⁻¹, as Hg, were prepared in methanol and kept refrigerated until use. Natural abundance MMHg working standard solutions of 1.993 and 2.042 µg ml⁻¹ were prepared by diluting the stock solutions with methanol. A 5.00 µg ml⁻¹ ethylmercury (EtHg) solution was prepared by diluting the stock solution in methanol for use as an internal standard.

¹⁹⁸Hg enriched MMHg spike solution at a nominal concentration of 0.55 µg ml⁻¹ in methanol was prepared from an isotopically enriched CH₃¹⁹⁸HgCl (MM¹⁹⁸Hg) stock (candidate CRM EOM-1) synthesized in our laboratory from commercially available inorganic ¹⁹⁸Hg (96% isotopic purity).⁶¹ From previous experience, although the uncertainty contribution from volume measurements is usually larger than that arising from mass, the overall uncertainty contributions from dilutions by volume remain insignificant compared with the total combined uncertainty characterising the overall procedure.⁶³ Thus, for simplicity of sample preparation, all dilutions were achieved by volume. Concentration of the spike solution was verified by reverse spike isotope dilution against the natural abundance MMHg standards.

The National Research Council Canada (NRCC, Ottawa,

Canada) biological CRMs DOLT-2 and DORM-2, as well as the new CRM DOLT-3, were used in the study.

Sample preparation and analysis procedure

Sample preparation was based on the procedure reported by Cai and Bayona.⁵ In brief, 0.25 g of sample with an appropriate amount of enriched MM¹⁹⁸Hg spike and 20 ml of 25% (m/v) methanolic KOH solution were shaken for 5 hours and then stored at 4 °C until analysis. Six reverse spike isotope dilution calibration samples were prepared to quantify the concentration of the enriched MM¹⁹⁸Hg spike. A 0.200 ml volume of enriched MM¹⁹⁸Hg spike solution and 0.240 ml of 2.042 µg ml⁻¹ (or 1.993 µg ml⁻¹) natural abundance MMHg solution were accurately pipetted into a vial and diluted with methanol to 10 ml. For SPME headspace sampling, only a 100 µl volume of digest or reverse spike isotope dilution calibration sample (due to the high sensitivity of SPME GC-ICP-MS) was transferred to a 50 ml glass vial for quantitation. After 20 ml of 1 mol l⁻¹ NaAc buffer solution and 1 ml of 1% NaBPr₄ were added, the vial was capped with a PTFE coated silicon rubber septum. The SPME needle was inserted through the septum and headspace sampling was performed for 10 min. During the extraction, the solution was vigorously stirred with a Teflon coated magnetic stir bar. The collected analyte was then desorbed from the SPME fiber onto the GC column. A 1 min desorption time at an injector temperature of 220 °C ensured complete desorption from the fiber.

Following injection of the sample onto the GC column, data acquisition on the ELAN 6000 was manually triggered. Isotopes of ²⁰²Hg, ²⁰⁰Hg and ¹⁹⁸Hg were monitored during every run. The mass bias correction solution (a 100 ng ml⁻¹ natural abundance MMHg standard) was introduced between samples to permit a mass bias correction factor to be determined. At the end of the chromatographic run, the acquired data were transferred to an off-line computer for further processing using in-house software to yield both peak height and peak area information. In this work, only peak areas were used to generate ²⁰⁰Hg/¹⁹⁸Hg or ²⁰²Hg/¹⁹⁸Hg ratios, from which the analyte concentrations in the biological samples were calculated.

Results and discussion

Optimization of GC-ICP-MS

Coupling of GC to ICP-MS, accomplished using a home-made interface and transfer line, and the optimization of the interface parameters, have been reported in detail previously.⁶² In brief, optimization of the ELAN 6000 and dead time correction were first performed as recommended by the manufacturer using a standard liquid sample introduction system. The plasma was then extinguished and the spray chamber and nebulizer assembly replaced with the transfer line and its adapter. The final optimization of lens voltage, RF power and Ar carrier gas flow for dry plasma conditions was performed by injection of 1 µl of a 50 ng ml⁻¹ propylated MMHg standard in isooctane.

The temperature of the GC injector is critical, since too high a temperature can lead to thermal decomposition of not only the mercury derivatives, but the SPME polymer coating as well. On the other hand, too low a temperature can result in inefficient desorption, leading to sample carry-over (memory) and extensive peak tailing. It was determined that a 1 min desorption time at a temperature of 220 °C assured the total desorption of the mercury species at the expense of a small decomposition of the mercury derivatives to yield a peak for Hg⁰ at 60 s in the chromatogram for standard solutions and samples, as shown in Fig. 1. The source of Hg⁰ was confirmed to be thermal decomposition of the derivatized Hg species, as indicated by the fact that ¹⁹⁸Hg was the dominant signal (as

Fig. 1 Chromatogram of a DOLT-3 extract with EtHg internal standard added obtained by SPME-GC-ICP-MS.

opposed to a natural abundance pattern if the procedural blank was the major source) at 60 s when using a 500 ng ml⁻¹ enriched MM¹⁹⁸Hg (96.2%) spike. All Hg species were well separated with little tailing of the peaks under the chosen experimental conditions, as is evident from Fig. 1.

Optimization of SPME sampling

Headspace sampling was chosen to minimize exposure of the SPME fibre to the sample matrix, thereby enhancing the lifetime of the fibre. SPME extraction can be influenced by a number of factors, including extraction temperature, extraction time, pH of the solution and the concentration of derivatization reagent. For simplicity, derivatization of Hg species and SPME sampling were performed at room temperature.

Equilibration time, which is the time required for the amount of analyte extracted to reach a steady-state, is one of the most important parameters influencing SPME extraction. At equilibrium, variations in the extraction time will not affect the amount of analyte extracted by the fibre, thereby providing the best measurement precision.⁴⁴ Thus, the effect of extraction time in the range of 2–30 min was investigated by using a 100 ng ml⁻¹ MMHg standard solution. It was found that an 8 min extraction time is required to achieve an equilibrium distribution of derivatized MMHg between the matrix and the PDMS fibre, as is shown in Fig. 2a. A 10 min extraction time was therefore chosen in this study to ensure complete equilibrium.

The effect of sample pH in the range of 1–8 was investigated. A high and constant response from a 100 ng ml⁻¹ MMHg standard solution was obtained over the range of pH 4–7, as is shown in Fig. 2b. Sensitivity decreased at both lower and higher pH. A pH of 5, obtained using a 1 mol l⁻¹ NaAc buffer solution, was chosen for simplicity in this study.

Use of NaBPr₄ instead of NaBEt₄ was chosen as the derivatization agent to permit use of EtHg as an internal standard for assessment of standard additions calibration. Furthermore, NaBPr₄ solution was found to be stable for at least 2 weeks when stored at 4 °C. The influence of NaBPr₄ concentration on SPME response was evaluated and no effect was detected in the concentration range from 0.2% to 2%. No NaBPr₄ concentrations higher than 2% were investigated based on an earlier report²⁸ that this could lead to self-ignition or degradation of the SPME fibre coating during the extraction

Fig. 2 Effects of extraction time and sample pH on SPME extraction: a, extraction time effect; b, pH effect.

procedure. A concentration of 1% NaBPr₄ was thus selected to ensure complete propylation of analyte in the real sample matrix.

It is of interest to determine the absolute amount of MMHg sampled by the SPME fiber under the chosen experimental conditions. The extraction efficiency of MMHg by the SPME PDMS fiber was estimated by a comparison of intensities arising from injection of a propylated MMHg standard in iso-octane with that arising from SPME sampling using a 100 ng ml⁻¹ standard solution. An extraction efficiency of 7.5% was obtained for SPME sampling of MMHg.

Application of isotope dilution and reverse-spike isotope dilution for the quantitation of MMHg in the new biological CRM DOLT-3

Determination of methylmercury is required for the certification of MMHg in a new biological dogfish liver CRM DOLT-3. As noted earlier, ID-ICP-MS is capable of compensating for any loss of analyte during sample derivatization and extraction, suppression of ion intensities by concomitant elements present in the sample matrix and for instrument drift, providing isotopic equilibrium is achieved between the added spike and the endogenous analyte in the sample. In addition, in order to achieve accurate and precise results, an interference free pair of isotopes must be available for ratio measurements, care must be taken to avoid any contamination during the process, and an optimum measurement procedure must be used to achieve accurate ratio measurements.

Equilibration between the added spike and the endogenous analyte in the sample is a prerequisite for achieving accurate results using isotope dilution techniques. Results would be biased low if equilibration between the spike and the sample was not achieved prior to the ratio measurements (with the spike being recovered with greater efficiency). In practice, validation of the achievement of equilibrium of the enriched spike and the sample is not easy. In an effort to ensure equilibration, spiked samples were digested with 25% methanolic KOH and stored in the dark at 4 °C for at least 2 d before ratio measurements. To verify the equilibration, ²⁰²Hg/¹⁹⁸Hg and ²⁰⁰Hg/¹⁹⁸Hg 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. This suggests that equilibration between the added spike and the endogenous MMHg in the sample has been achieved prior to the derivatization reaction.

Mass bias corrected ratios of 2.998 ± 0.048 and 2.315 ± 0.033 (one standard deviation, $n = 3$) obtained in unspiked DOLT-3 are not significantly different from the expected natural abundance ratios of 2.996 and 2.317 for ²⁰²Hg/¹⁹⁸Hg and ²⁰⁰Hg/¹⁹⁸Hg, respectively. This confirmed that no significant spectroscopic interference on any of the three isotopes arises from the sample matrix, permitting accurate results to be obtained using either isotope pair.

Mass bias correction can be carried out either by injection of natural MMHg standard or using the inorganic Hg²⁺ peak in the chromatogram of each sample. The latter approach would not only save analysis time but would also provide better mass bias correction since instrument drift is accounted for with each sample. Unfortunately, the enriched MM¹⁹⁸Hg spike contains a small amount of inorganic ¹⁹⁸Hg²⁺ which thus alters the ²⁰²Hg/¹⁹⁸Hg and ²⁰⁰Hg/¹⁹⁸Hg ratios from their expected natural ratios in the spiked DOLT-3. To achieve best accuracy and precision for the ratio measurement, the first approach of repeated injections of the mass bias correction standard between spiked samples and reverse spike ID samples was performed. The mass bias correction factors obtained were 1.064 ± 0.026 and 1.046 ± 0.024 (mean and one standard deviation, $n = 8$) for ²⁰²Hg/¹⁹⁸Hg and ²⁰⁰Hg/¹⁹⁸Hg, respectively, indicating no significant mass bias drift during a run sequence. The ²⁰⁰Hg/¹⁹⁸Hg isotope pair was used for final quantitation of MMHg in DOLT-3 because the average mass bias correction factor for this isotope pair was smaller.

The following equation was used for the quantitation of MMHg in DOLT-3:

$$C_x = C_z \times \frac{v_y}{w \times m_x} \times \frac{v_z}{v'_y} \times \frac{A_y - B_y \times R_n}{B_{xz} \times R_n - A_{xz}} \times \frac{B_{xz} \times R'_n - A_{xz}}{A_y - B_y \times R'_n} - C_b \quad (1)$$

where C_x is the blank corrected MMHg concentration as Hg (μg g⁻¹) based on dry mass, C_z is the concentration of natural abundance MMHg standard (μg ml⁻¹), v_y is the volume (ml) of spike used to prepare the blend solution of sample and spike, m_x is the mass (g) of sample used, w is the dry mass correction factor, v_z is the volume (ml) of natural abundance MMHg standard used, v'_y is the volume (ml) of spike used to prepare the blend solution of spike and natural abundance MMHg standard solution, A_y is the abundance of the reference isotope (²⁰⁰Hg) in the spike, B_y is the abundance of the spike isotope (¹⁹⁸Hg) in the spike, A_{xz} is the abundance of the reference isotope in the sample or in the standard, B_{xz} is the abundance of the spike isotope in the sample or in the standard, R_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike, R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and natural abundance MMHg standard and C_b is the blank concentration (μg g⁻¹). An MMHg concentration of 1.540 ± 0.025 μg g⁻¹ (one standard deviation, $n = 6$) as mercury was obtained in DOLT-3.

An average concentration of 0.0711 ± 0.0007 μg g⁻¹ (one standard deviation, $n = 3$), obtained from three sample blanks, is insignificant compared to the MMHg concentration in DOLT-3, confirming that contamination was effectively under control during sample preparation. This blank was subtracted from the gross MMHg concentration measured in DOLT-3.

The detection limit for the ID SPME GC-ICP-MS technique was calculated based on the response from three MM¹⁹⁸Hg spiked blank measurements. A value of 2.1 ng g⁻¹ was estimated, based on three times the standard deviation of measured concentrations normalized to a 0.25 g subsample and

derivatization of a 0.10 ml volume of the methanolic KOH extract.

Validation of isotope dilution SPME GC-ICP-MS method for the determination of MMHg

Two biological CRMs, DORM-2 and DOLT-2, with certified values for MMHg were used to validate the proposed method. Concentrations of 4.484 ± 0.029 and $0.719 \pm 0.015 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$) as mercury were obtained using ID-SPME-GC-ICP-MS for DORM-2 and DOLT-2, respectively, in good agreement with their certified values of 4.47 ± 0.32 and $0.693 \pm 0.053 \mu\text{g g}^{-1}$ (as 95% confidence interval).

Results for MMHg in DORM-2 using standard additions calibration with SPME-GC-ICP-MS

A subsequent comparative analysis of DORM-2 fish tissue was undertaken using the method of standard additions for calibration with SPME-GC-ICP-MS. Additions of approximately 1- and 2-fold of the expected MMHg concentration in the DORM-2 were made. A spike of 1000 ng of EtHg was added to all samples as an internal standard. Quantitation of MMHg was based on the ^{202}Hg intensity ratios obtained from the peak area of the MMHg divided by the peak area for the EtHg. The correlation coefficient for the standard additions calibration curve for MMHg in the concentration range of $0\sim 9 \mu\text{g g}^{-1}$ was 0.9998. A mean concentration of $4.69 \pm 0.12 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$) was obtained, in good agreement with the certified value.

It is of interest to compare this result with that generated without using EtHg as an internal standard wherein quantitation of MMHg is based solely on the peak area of MMHg. A mean concentration of $4.67 \pm 0.36 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$) was obtained, in good agreement with the certified value. As expected, the precision of 7.7% RSD obtained using standard additions calibration is significantly poorer than the precision of 2.5% RSD obtained using standard additions calibration with EtHg as the internal standard. For this study, EtHg served as an effective internal standard for determination of MMHg in biological samples, thereby enhancing the precision of the SPME sampling technique. Other studies have suggested that EtHg may degrade, depending on extraction conditions.^{64,65}

Conclusion

A rapid, accurate and precise method has been developed for the determination of MMHg in biological samples using ID-SPME-GC-ICP-MS. A significant nearly 4-fold improvement in the precision of MMHg determination in DORM-2 using ID (0.65% RSD), as opposed to standard additions calibration (2.5% and 7.7% RSD with and without EtHg internal standard, respectively) was obtained, clearly demonstrating its superior capability in overcoming the generally poor precision associated with the SPME sampling technique. The proposed method has a sufficiently good detection limit (2.1 ng g^{-1}) to make it well suited to the certification of MMHg in the new CRM DOLT-3. To the best of our knowledge, this is the first report of the use of the isotope dilution technique to overcome the poor precision associated with SPME sampling using GC separation and ICP-MS detection for determination of MMHg in biological samples.

References

- 1 P. J. Craig, *Organometallic Compounds in the Environment: Principles and Reactions*, Longman, Harlow, 1986.
- 2 N. S. Bloom, *Can. J. Fish. Aquat. Sci.*, 1989, **46**, 1131–1140.

- 3 L. Liang, M. Horvat, E. Cernichiari, B. Gelein and S. Balogh, *Talanta*, 1996, **43**, 1883–1888.
- 4 R. Fischer, S. Rapsomanikis and M. O. Andreae, *Anal. Chem.*, 1993, **65**, 763–766.
- 5 Y. Cai and J. M. Bayona, *J. Chromatogr. A*, 1995, **696**, 113–122.
- 6 P. Grinberg, R. C. Campos, Z. Mester and R. E. Sturgeon, *Spectrochim. Acta, Part B*, 2002, in the press.
- 7 P. Quevauviller, O. Donard, J. C. Wasserman, F. Martin and J. Schneider, *Appl. Organomet. Chem.*, 1992, **6**, 221–228.
- 8 M. Hempel, H. Hintelmann and R. D. Wilken, *Analyst*, 1992, **117**, 669–672.
- 9 K. C. Bowles and S. C. Apte, *Anal. Chem.*, 1998, **70**, 395–399.
- 10 M. Horvat, N. S. Bloom and L. Liang, *Anal. Chim. Acta*, 1993, **281**, 135–152.
- 11 M. Horvat, V. Mandic, L. Liang, N. S. Bloom, S. Padberg, Y. H. Lee, H. Hintelmann and J. M. Benoit, *Appl. Organomet. Chem.*, 1994, **8**, 533–540.
- 12 M. Horvat, L. Liang and N. S. Bloom, *Anal. Chim. Acta*, 1993, **282**, 153–168.
- 13 M. A. Morrison and J. H. Weber, *Appl. Organomet. Chem.*, 1997, **11**, 761–769.
- 14 H. Emteborg, E. Bjorklund, F. Odman, L. Karlsson, L. Mathiasson, W. Frech and D. C. Baxter, *Analyst*, 1996, **121**, 19–29.
- 15 M. J. Vazquez, A. M. Carro, R. A. Lorenzo and R. Cela, *Anal. Chem.*, 1997, **69**, 221–225.
- 16 C. M. Tseng, A. De Diego, F. M. Martin and O. F. X. Donard, *J. Anal. At. Spectrom.*, 1997, **12**, 629–635.
- 17 C. M. Tseng, A. De Diego, J. C. Wasserman, D. Amouroux and O. F. X. Donard, *Chemosphere*, 1999, **39**, 1119–1136.
- 18 R. G. Fernandez, M. M. Bayon, J. I. G. Alonso and A. Sanz-Medel, *J. Mass Spectrom.*, 2000, **35**, 639–646.
- 19 H. Emteborg, J. Snell, J. Qian and W. Frech, *Chemosphere*, 1999, **39**, 1137–1152.
- 20 N. G. Orellana-Velado, R. Pereiro and A. Sanz-Medel, *J. Anal. At. Spectrom.*, 1998, **13**, 905–909.
- 21 S. Hanstrom, C. Briche, H. Emteborg and D. C. Baxter, *Analyst*, 1996, **121**, 1657–1663.
- 22 S. Rapsomanikis and P. J. Craig, *Anal. Chim. Acta*, 1991, **248**, 563–567.
- 23 L. Lambertsson, E. Lundberg, M. Nilsson and W. Frech, *J. Anal. At. Spectrom.*, 2001, **16**, 1296–1301.
- 24 R. C. Rodríguez Martín-Doimeadios, E. Krupp, D. Amouroux and O. F. X. Donard, *Anal. Chem.*, 2002, **74**, 2505–2512.
- 25 S. Diez and J. M. Bayona, *J. Chromatogr. A*, 2002, **963**, 345–351.
- 26 Y. Cai, S. Monsalud, R. Jaffe and R. D. Jones, *J. Chromatogr. A*, 2000, **876**, 147–155.
- 27 P. Grinberg, R. C. Campos, Z. Mester and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2003, submitted for publication.
- 28 M. Abuin, A. M. Carro and R. A. Lorenzo, *J. Chromatogr. A*, 2000, **889**, 185–193.
- 29 Y. Cai, S. Monsalud and K. G. Furton, *Chromatographia*, 2000, **52**, 82–86.
- 30 G. L. Hu and X. R. Wang, *Anal. Lett.*, 1998, **31**, 1445.
- 31 G. L. Hu, X. R. Wang, Y. R. Wang, C. Xi and J. Li, *Anal. Lett.*, 1997, **30**, 2579–2594.
- 32 H. E. L. Palmieri and L. V. Leonel, *Fresenius' J. Anal. Chem.*, 2000, **366**, 466–469.
- 33 R. Rodil, A. M. Carro, R. A. Lorenzo, M. Abuin and R. Cela, *J. Chromatogr. A*, 2002, **963**, 313–323.
- 34 L. Liang, M. Horvat and N. S. Bloom, *Talanta*, 1994, **41**, 371–379.
- 35 R. Ritsema and O. F. X. Donard, *Appl. Organomet. Chem.*, 1994, **8**, 571–575.
- 36 J. Holz, J. Kreutzmann, R. D. Wilken and R. Falter, *Appl. Organomet. Chem.*, 1999, **13**, 789–794.
- 37 M. Caricchia, G. Minervini, P. Soldati, S. Chiavarini, C. Ubaldi and R. Morabito, *Microchem. J.*, 1997, **55**, 44–55.
- 38 R. Pereiro, A. Wasik and R. Łobiński, *J. Chromatogr. A*, 1998, **795**, 359–370.
- 39 C. Gerbersmann, M. Heisterkamp, F. C. Adams and J. A. C. Broekaert, *Anal. Chim. Acta*, 1997, **350**, 273–285.
- 40 Q. Tu, J. Qian and W. Frech, *J. Anal. At. Spectrom.*, 2000, **15**, 1583–1588.
- 41 S. Tutschku, M. M. Schantz and S. A. Wise, *Anal. Chem.*, 2002, **74**, 4694–4701.
- 42 S. Slaets, F. Adams, I. R. Pereiro and R. Łobiński, *J. Anal. At. Spectrom.*, 1999, **14**, 851–857.
- 43 H. Tao, T. Murakami, M. Tominaga and A. Miyazaki, *J. Anal. At. Spectrom.*, 1998, **13**, 1085–1093.
- 44 C. Arthur and J. Pawliszyn, *Anal. Chem.*, 1990, **62**, 2145–2148.
- 45 J. Pawliszyn, *Solid Phase Microextraction: Theory and Practice*, Wiley, 1997.

- 46 C. Haberhauer-Troyer, M. Crnoja, E. Rosenberg and M. Grasserbauer, *Trends Anal. Chem.*, 2000, **366**, 329–331.
- 47 Z. Mester, R. E. Sturgeon and J. Pawliszyn, *Spectrochim. Acta, Part B*, 2001, **56**, 233–260.
- 48 A. Beichert, S. Padberg and B. W. Wenclawiak, *Appl. Organomet. Chem.*, 2000, **14**, 493–498.
- 49 Y. Cai, S. Monsalud, K. G. Furton, R. Jaffe and R. D. Jones, *Appl. Organomet. Chem.*, 1998, **12**, 565–569.
- 50 B. He, G. B. Jiang and Z. M. Ni, *J. Anal. At. Spectrom.*, 1998, **13**, 1141–1144.
- 51 P. de Bièvre in *Trace Element Analysis in Biological Specimens*, ed. R. F. M. Herber and M. Stoeppler, Elsevier, 1994.
- 52 Report of the 5th meeting of the Comité Consultatif pour la Quantité de Matière, BIPM, February, 1999.
- 53 H. Hintelmann, R. Falter, G. Ilgen and R. D. Evans, *Fresenius' Anal. Chem.*, 1997, **358**, 363–370.
- 54 M. Barshick, S. A. Barshick, E. B. Walsh, M. A. Vance and P. F. Britt, *Anal. Chem.*, 1999, **71**, 483–488.
- 55 N. Demuth and K. G. Heumann, *Anal. Chem.*, 2001, **73**, 4020–4027.
- 56 I. Gelaude, R. Dams, M. Resano, F. Vanhaecke and L. Moens, *Anal. Chem.*, 2002, **74**, 3833–3842.
- 57 H. Hintelmann and R. D. Evans, *Fresenius' Anal. Chem.*, 1997, **358**, 378–385.
- 58 J. Qvarnström and W. Frech, *J. Anal. At. Spectrom.*, 2002, **17**, 1486–1491.
- 59 R. C. Rodríguez Martín-Doimeadios, T. Stoichev, E. Krupp, D. Amouroux, M. Holeman and O. F. X. Donard, *Appl. Organomet. Chem.*, 2002, **16**, 610–615.
- 60 J. P. Snell, I. Stewart, R. E. Sturgeon and W. Frech, *J. Anal. At. Spectrom.*, 2000, **15**, 1540–1545.
- 61 C. Bancon-Montigny, L. Yang, V. Columbini, Z. Mester and R. E. Sturgeon, *Anal. Chim. Acta*, 2003, submitted for publication.
- 62 L. Yang, Z. Mester and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2002, **17**, 944–949.
- 63 L. Yang, Z. Mester and R. E. Sturgeon, *Anal. Chem.*, 2002, **74**, 2968–2976.
- 64 E. Bulska, H. Emteborg, D. C. Baxter, W. Frech, D. Ellingsen and Y. Thomassen, *Analyst*, 1992, **117**, 657–663.
- 65 H. Emteborg, N. Hadgu and D. C. Baxter, *J. Anal. At. Spectrom.*, 1994, **9**, 297–303.