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# Direct Determination of Methylmercury and Inorganic Mercury in Biological Materials by Solid Sampling-Electrothermal Vaporization-Inductively Coupled Plasma-Isotope Dilution-Mass Spectrometry

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**This paper reports on the use of solid sampling-electrothermal vaporization-inductively coupled plasma mass spectrometry (SS-ETV-ICPMS) for the direct and simultaneous determination of methylmercury and inorganic mercury in biological materials. The main advantage of this fast and sensitive method is that no sample preparation is required. In this way, the sample throughput can be considerably increased, problems of contamination and analyte losses are kept to a minimum and, even more important, the original chemical form of the different analyte species in the solid samples is preserved. To achieve this goal, a solid sample is inserted into a graphite furnace of the boat-in-tube type and is subsequently submitted to an appropriate temperature program, leading to the separate vaporization of methylmercury and inorganic mercury, which are transported into the ICP by means of an argon carrier gas. The separation was accomplished within 75 s. For the quantification of the two peaks, species-unspecific isotope dilution was used. For this purpose, a stable flow of argon loaded with gaseous Hg isotopically enriched in $^{200}$Hg was generated using a permeation tube that was constructed in-house. Its emission rate was determined by collecting the mercury released during a given time interval on a gold-coated silica absorber, after which the amount collected was released by heating of the absorber and determined by cold vapor atomic absorption spectrometry (CVAAS) and cold vapor atomic fluorescence spectrometry (CVAFS). A reference material from the Canadian National Research Council (NRC) (TORT-2) was used to assess the accuracy of the method. For the application of the method to samples with diverse mercury contents, the spike/sample ratio can be optimized by varying the emission rate of the permeation tube simply by adapting its temperature. To prove the feasibility of this approach, two reference materials (BCR 463 and DORM-2) with a methylmercury content more than 10 times higher than that of TORT-2 were also analyzed. The detection limits obtained for 1 mg of sample (2 ng g$^{-1}$ and 6 ng g$^{-1}$ for methylmercury and inorganic mercury, respectively) were found to be sufficiently low for this kind of application and are competitive when compared to other techniques.**

Mercury is ubiquitous in the environment and exists in several chemical and physical forms with different properties and toxicity. The most toxic compound, methylmercury, is mainly formed in the aquatic environment by biotic or abiotic processes.[1] Because of its ability to bioaccumulate in fish tissues and its extreme toxicity, considerable effort and progress have been made in the development of techniques capable of separating, identifying, and quantifying various mercury species in this kind of samples.

Most common methods used, such as gas chromatography (GC) coupled to atomic absorption spectrometry (AAS),[2−4] atomic fluorescence spectrometry (AFS),[5−7] (microwave induced plasma) atomic emission spectrometry (MIP)AES,[8−10] electron capture detection (ECD)[11] and inductively coupled plasma mass spectrometry (ICPMS),[10,12] and high performance liquid chromatography (HPLC) coupled to AFS[13,14] and ICPMS,[15] require extraction or derivatization or both of the target compounds (most often with $NaBEt_4$) before separation and detection, which can result in species transformation and, therefore, lead to significant errors.[16−19]

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Alternatively, electrothermal atomization/vaporization-based methods have shown some potential to separate species according to their volatility. Appearance of two peaks due to different species has been observed.[20,21] However, quantitative results have seldom been reported. One of the main problems for obtaining reliable quantitative information is achieving proper calibration of all species involved, and thus, less straightforward two-step approaches based on the use of two different temperature programs (one for determination of the total content and another for determination of the less volatile species after sample pretreatment and selective removal at low temperatures of the more volatile one) have been proposed.[22−24] On other occasions, only semiquantitative results were obtained.[25] Another aspect worth noting is that although it is well-known that GFAAS or ETV-ICPMS are compatible with the direct analysis of solid samples, to the best of our knowledge, no attempt has been made to study the possibilities of direct speciation analysis of solid samples using these methods.

The use of direct solid sampling methods, and particularly of solid sampling-ETV-ICPMS, could open possibilities for the direct speciation of mercury in environmental samples. This method combines the detection power of ICPMS (high sensitivity, isotopic capabilities) with all of the advantages of direct solid sampling analysis (high sample throughput, reduced risk of losses and contamination, low sample mass requirement). Furthermore, it is particularly attractive for speciation studies that the original chemical form of the different analyte species in the sample can be preserved, because no chemical reagents that might disturb the interspecies equilibrium need to be added to the sample (it is fair to indicate that the temperature program applied to the sample might increase the risk of species transformation; however, as will be proved in this work, this factor does not seem to be significant, at least for the samples studied).

In this work, the possibilities of solid sampling-ETV-ICPMS for the direct quantification of both inorganic and methylmercury in biological materials in a single tube firing were evaluated. A representative reference material from the Canadian National Research Council (NRC) (TORT-2, lobster hepatopancreas) was selected for the optimization study. Different calibration approaches (direct calibration, single standard addition, isotope dilution) as well as different possibilities for overcoming the effect of the matrix on the plasma conditions will be discussed. To further prove the flexibility of the method to deal with samples with diverse mercury contents, two reference materials (BCR CRM 463, tuna fish and DORM-2, dogfish muscle) with a concentration >10 times higher than that of the TORT-2 were also analyzed. Finally, the detection limits were calculated and compared with literature values.

## EXPERIMENTAL SECTION

**Instrumentation.** *ETV-ICPMS.* A commercially available graphite furnace of the "boat-in-tube" type (SM-30, Grün Analytische Mess-Systeme GmbH) coupled to a Perkin-Elmer Sciex Elan 5000 ICP-mass spectrometer was used. This ETV device was originally developed for use with GFAAS. The modifications necessary to connect this type of furnace to the ICPMS have been described elsewhere.[26] The graphite sample holders ("boats") can be easily and reproducibly loaded into the cylindrical graphite furnace with the aid of a pair of tweezers, sliding on a rail, which is rigidly mounted in front of the furnace. The temperature of the furnace can be programmed by means of in-house-developed computer software and is monitored using an optical pyrometer (PY, Grün Optik, Germany). The flow rate of the Ar carrier gas was controlled by means of a mass flow controller (Brooks Instruments B. V., model 5876, The Netherlands).

*AAS and AFS.* The atomic absorption spectrometer used is a Mercury Monitor 3200 (LDC Analytical Inc., Thermo Instruments Inc.), which consists of a double-beam photometer (path length of cell, 10 cm), a Hg lamp with interference filter, and a double photon detector. The selected wavelength was 253.7 nm.

The atomic fluorescence spectrometer used is a Merlin PSA 10023 (PSA Analytical, Kemsig, Sevenoaks, U.K.), which consists of an open chimney, a reference cell, a high-intensity Hg lamp with the interference filter under an angle of 90° and a photon detector. The selected wavelength was 253.7 nm.

The spectrometers were coupled on-line by connecting the outlet of the AAS instrument to the inlet of the AFS instrument by Tygon tubing. In this way, the absorption and fluorescence signals could be quasisimultaneously measured for each sample. By means of the appropriate software, the transient signals were recorded, and net peak areas were calculated.

*Other Equipment.* A microbalance (Sartorius M3P, Germany) with a readability of 1 $\mu$g was used for weighing the samples.

**Standards and Samples.** *Standards.* Water was doubly distilled and further purified using a Milli-Q water purification system (Millipore). $HNO_3$ was purified by subboiling distillation in quartz equipment. A standard solution of 1 g $L^{-1}$ $Hg^{2+}$ was prepared by dissolving the appropriate amount of $HgCl_2$ (Carlo Erba, Italy) in 0.14 M $HNO_3$ or Milli-Q water. The 1 g $L^{-1}$ MeHgCl standard was prepared by dissolving the appropriate amount of MeHgCl (Merck − Schuchardt) in ethanol (Panreac, Spain) or Milli-Q water. Further dilutions down to 10 and 15 $\mu$g $L^{-1}$ were carried out daily.

TAC (thioacetamide) solution was prepared by dissolving the appropriate amount of TAC (Riedel-de Haën, Germany) in Milli-Q water to obtain a solution of 60 $\mu$g $L^{-1}$. TMAH (tetramethylammonium hydroxide) solution (10%) was obtained from UCB, Belgium.

*Samples.* The samples studied (certified reference materials) were obtained from the Canadian National Research Council (TORT-2, lobster hepatopancreas and DORM-2, dogfish muscle) and from the Community Bureau of Reference (BCR CRM 463

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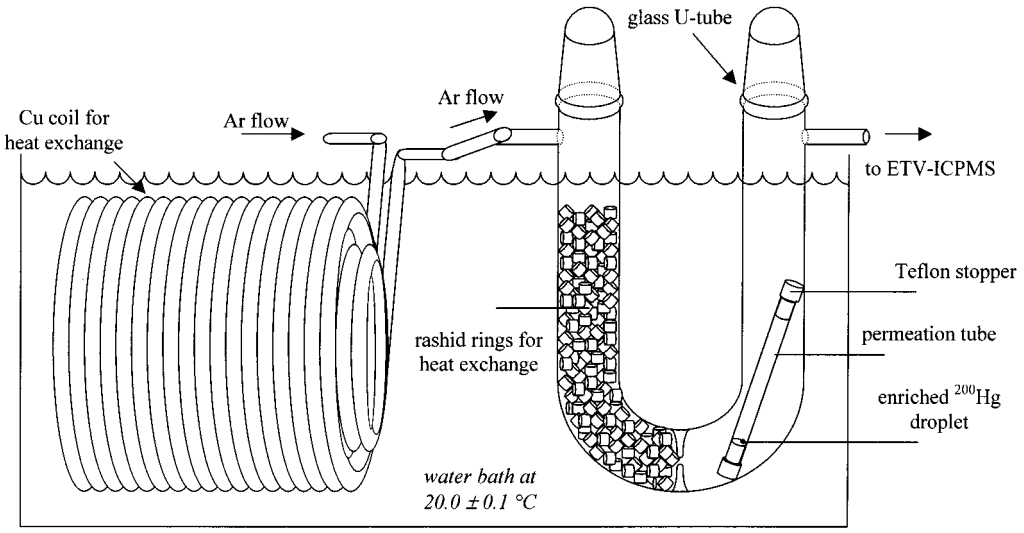


Figure 1. Equipment for generation of the $^{200}$Hg-enriched spike.

Table 1. Instrument Settings and Acquisitions Parameters Used[a]

| | |
|---|---|
| rf power (W) | 1100 |
| gas flow rates (L min$^{-1}$) | |
| carrier | 0.9 |
| plasma | 15 |
| auxiliary | 1.2 |
| sampling depth (mm) | 10 |
| sampling cone | nickel; 1.0-mm aperture diameter |
| skimmer | nickel; 0.75-mm aperture diameter |
| lens voltages (V)[b] | P: −66.3; B: +10.5; S2: −7.8; E1: + 4.2 |
| dead time (ns) | 69 |
| Hg Determinations in Sample | |
| scanning mode | peak hop transient |
| dwell time (ms) | 30 |
| sweeps/reading | 1 |
| readings/replicate | 1200 (825)[c] |
| points/spectral peak | 1 |
| signals monitored | $^{200}Hg^+$, $^{202}Hg^+$, ($^{80}Ar_2^+$) |

[a] Perkin-Elmer Sciex Elan 5000. [b] Optimized using pneumatic nebulization; no further tuning required when switching to ETV. [c] When $^{80}Ar_2^+$ is also monitored.

tuna fish). All materials are available in powdered form. No sample pretreatment, except for shaking the bottle containing the sample for several minutes for homogenization prior to analysis, was carried out.

**Procedure for Solid Sampling-ETV-ICPMS.** The samples (between 1 and 1.5 mg) were weighed directly into the graphite sample boat, which was subsequently inserted into the furnace for the analysis. The operating conditions are summarized in Table 1. The optimized temperature program used is given in Table 2.

**Procedure for the Generation of the Enriched Hg Spike for Isotope Dilution.** The permeation tube emitting Hg was produced in-house. It consists of a polyethylene tube (4.15-mm i.d.; 6.4-mm o.d.; length, 4.6 cm) closed with 2 Teflon stoppers. A droplet of isotopically enriched metallic Hg (96.736% $^{200}$Hg) (Eurisotop, France) was mixed with a smaller amount of metallic Hg of natural isotopic composition and inserted in the tube (total weight, ∼10 mg). The permeation tube was inserted in a glass U-tube (Figure 1) and placed in a thermostated waterbath (±0.1 °C) (MP-5, Julabo Labortechnik GmbH).[27,28]

Ar gas was sent through a copper coil (copper tube with 4 mm i.d., 6 mm o.d., length ∼10 m, wound as a coil with a diameter of 8 cm inside a coil with a diameter of 10 cm), also immersed in the waterbath and connected to the glass tube, to transport the generated Hg vapor to the graphite furnace first and to the ICP mass spectrometer afterward. By guiding the spike through the furnace first, it is subjected to the temperature program and, thus, is exposed to the same conditions as the sample. The copper coil is used for a better heat exchange, since the emission rate of the permeation tube is strongly dependent on the temperature.

**Safety Note**: Mercury is toxic by inhalation; danger to health exists if there is an accumulation in the body.

## RESULTS AND DISCUSSION

**Volatilization of Mercury Species.** The reference material TORT-2 (lobster hepatopancreas) was selected as a representative sample to examine the possibilities of solid sampling-ETV-ICPMS for speciation. In this sample, the contents of methylmercury and total mercury are certified. The difference in volatility between methylmercury species and inorganic mercury was sufficient for selective vaporization. After careful optimization of the temperature program (Table 2), two separate peaks could be clearly observed (see Figure 2a). The first one appeared during the first vaporization step (at 200 °C) and corresponds to methylmercury; the second one appeared during the second vaporization step (400−700 °C) and was attributed to inorganic mercury. The identity of these peaks was verified by using another reference material (DORM-2) in which mercury is present as methylmercury only.

In Figure 2a, the beginning of both vaporization steps is accompanied by an effect on the Ar-dimer signal.[29] More important, the monitoring of the $^{80}Ar_2^+$ signal also indicates that, although for the first peak no signal suppression effect is seen, for the second, the situation is different. Evidence of suppression

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Table 2. Optimized Temperature Program[a]

| step | duration step (s) | temperature (°C) | description step |
|---|---|---|---|
| vaporization (methylmercury) | 17 | 150 → 200 | rapid heating to 150 °C, ramp heating from 150 to 200 °C, 5 s at 200 °C |
| intermediate | 15 | 20 | no power applied, cooling, allowing a good separation between two peaks and minimizing risk of species transformation |
| vaporization (inorg. Hg) | 22 | 400 → 700 | rapid heating to 400 °C, ramp heating from 400 to 700 °C, 5 s at 700 °C |
| intermediate | 15 | 20 | no power applied, cooling and switching valve to vent position |
| cleaning | 3 | 2400 | cleaning of furnace |
| intermediate | 10 | 20 | no power applied, cooling |
| cleaning | 3 | 2400 | cleaning furnace |

[a] Neither drying nor pyrolysis step were used to prevent losses of volatile mercury species.

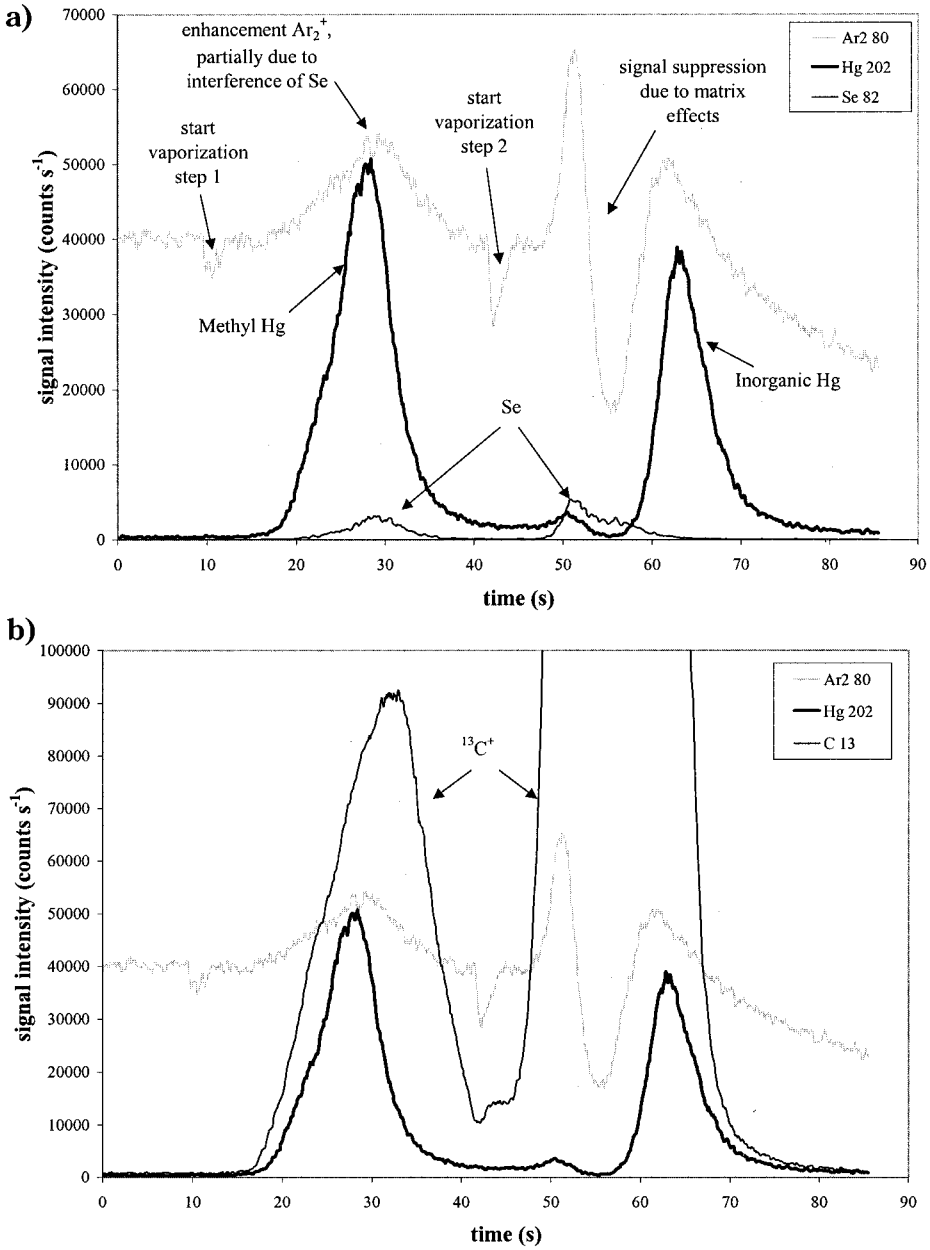


Figure 2. Separation of methylmercury and inorganic Hg in TORT-2 (1.079 mg). Monitoring of (a) $^{80}Ar_2^+$, $^{202}Hg^+$, and $^{82}Se^+$ and (b) $^{80}Ar_2^+$, $^{202}Hg^+$, and $^{13}C^+$.

on the dimer signal is clear.[30] The suppression of the inorganic mercury signal,at the very moment its vaporization is started can also be observed. This indicates that methylmercury, because of its volatility, is vaporized before most of the organic matrix is released. However, for the inorganic Hg, it was not possible to achieve the same favorable situation. The monitoring of the $^{13}C^+$

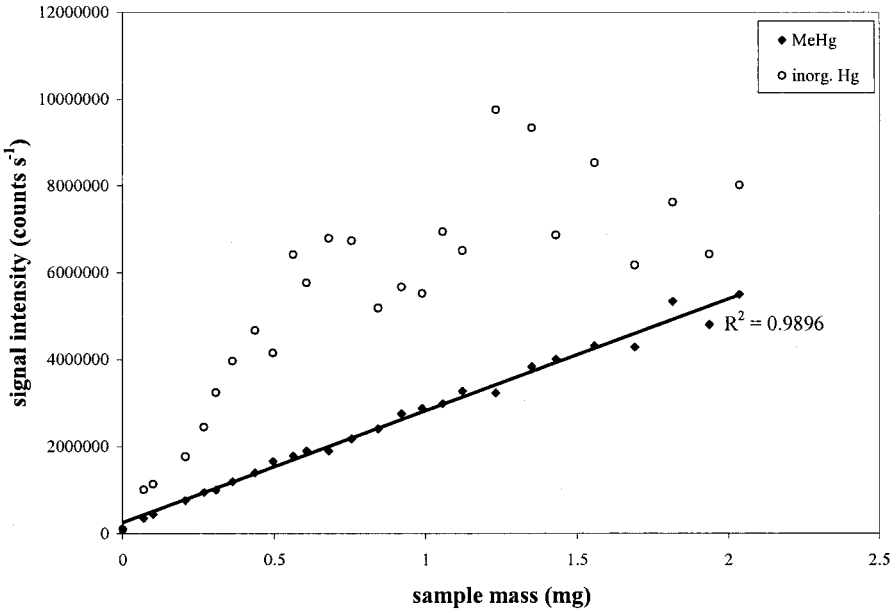


Figure 3. Mass response curves for methylmercury and inorganic Hg in TORT-2.

signal confirmed this hypothesis (Figure 2b). On the other hand, an increase in the $^{80}Ar_2^+$ signal during the first vaporization step and also during the second just before the suppression can be seen in Figure 2a, as well. This effect could to a large extent be explained by interference from $^{80}Se^+$, because volatile Se compounds are present in the sample (note that the abundance of the Se isotope monitored in Figure 2a, $^{82}Se^+$, is five times lower than that of the interfering isotope, $^{80}Se^+$). Enhancement in the ionization efficiency by introducing carbon-containing compounds has also been described and could play an additional role to explain this increase in signal intensity.[31−34]

The evolution of the signal with sample mass is shown in Figure 3. Although for methylmercury, good linearity is attainable, for inorganic Hg, deviation from linearity is evident. This could be expected, considering the signal suppression caused by the matrix, and is a problem that has to be tackled in order to obtain a reliable quantification.

**Evaluation of Calibration Methods.** It is clear that it is necessary to find a calibration method suitable for the separate quantification of both species and capable of correcting for the matrix effects observed for the second peak. To do so, different strategies were evaluated.

Standard addition using a solution of the analyte proved successful in similar situations in which matrix effects in solid sampling-ETV-ICPMS had to be corrected for.[35−37] Different possibilities were explored, and MeHgCl and $HgCl_2$ standard solutions in Milli-Q water, in 0.14 M $HNO_3$, and in ethanol were prepared and tested; however, results were unsatisfactory. The main problem for the addition of a standard is that because of the volatility of methylmercury, it is not possible to use a drying step at a sufficiently high temperature without losing the analyte. As a result, the remaining (not vaporized) solvent produced a suppression effect during the first vaporization step that did not appear when the solid sample was vaporized alone. Similar results were observed when adding the pure solvents to the sample (see $Ar_2^+$ signal in Figure 4). This effect was already pointed out by Willie et al.[22] and poses a serious problem for calibration of the signal. It is also interesting to indicate that the vaporization of the standards (without sample) yielded hardly any signal, which is probably not only due to the signal suppression, but also a result of the absence of a physical carrier to transport the mercury to the plasma.[22,38]

Another possibility tested was the use of chemical modifiers, despite the fact that interaction of the chemical modifiers with the analyte species may affect the chemical form in which these species are present in the sample. Attempts were made to carry out in situ microdigestion with TAC[39] and with TMAH,[22] the latter frequently applied when alkaline digestion is performed. Additionally, in these experiments, the presence of the solvent caused insurmountable problems.

The addition of an internal standard often is appropriate for correction of matrix effects in solid sampling-ETV-ICPMS.[36,37] The conditions that an element has to fulfill to be an efficient internal standard were summarized in a previous paper:[36] (a) the element should have a mass-to-charge ratio and an ionization potential similar to that of the analyte and (b) the element should exhibit a similar furnace behavior. Unfortunately, no element totally mimics the furnace and plasma behavior of Hg. Alternatively, the

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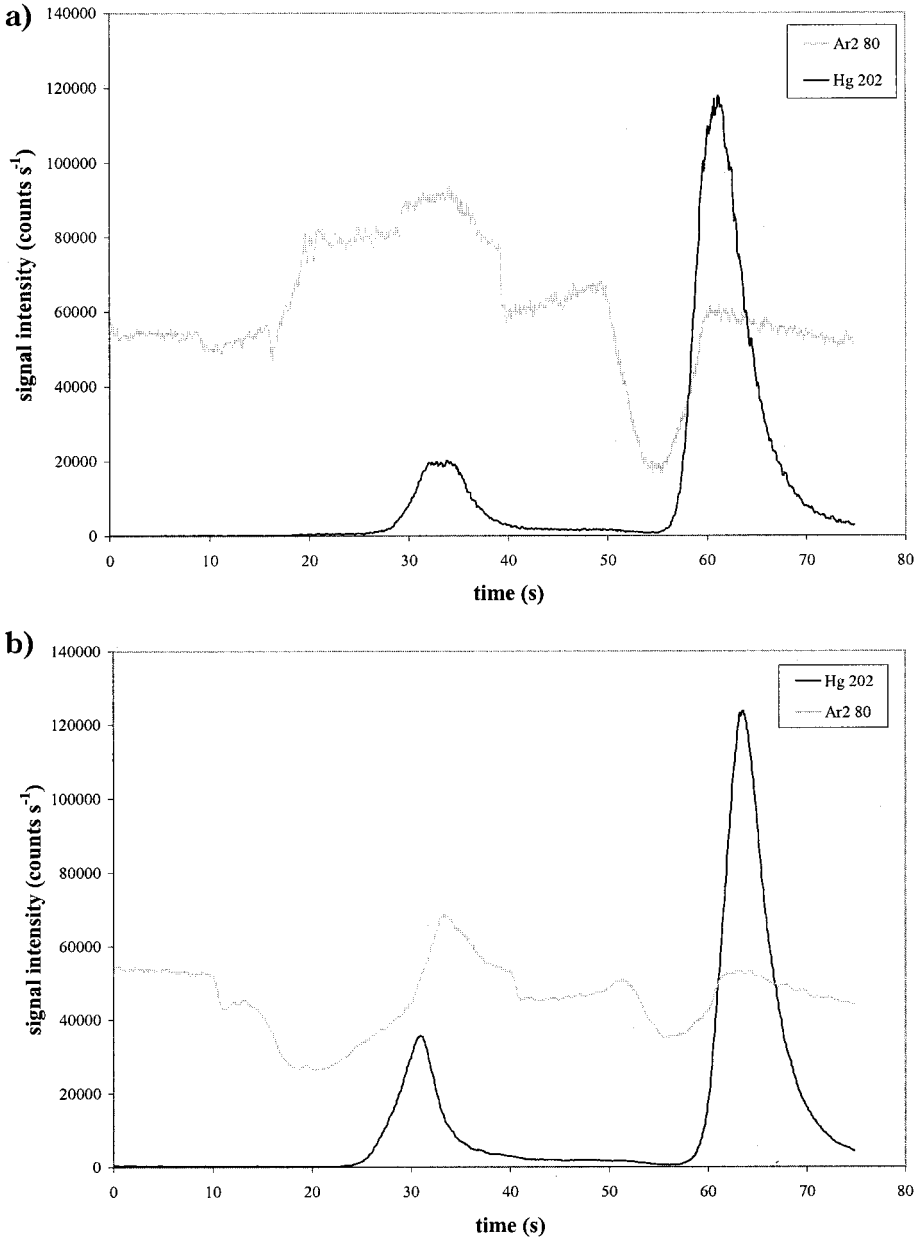


Figure 4. Influence of addition of (a) Milli-Q water and (b) ethanol on the Hg and Ar dimer signal profiles for TORT-2 ((a) 0.980 mg and (b) 1.148 mg).

use of the $Ar_2^+$ signal as internal standard to mathematically correct for suppression of the analyte signal has been reported on.[30,40] This approach was also evaluated, but the results were not satisfactory. This could be explained by the overlap of the signals of $^{80}Se^+$ and $^{80}Ar_2^+$ (already commented on earlier) in addition to the large difference in mass-to-charge ratio between Hg and the argon dimer.

In conclusion, it appears that the only potentially successful approaches are (a) Calibration with matrix-matched solid standards. However, this approach has serious disadvantages, because the method will hardly be applicable to real samples (for which finding a reference material that matches the sample is not always possible), whereas the uncertainty of the Hg content of the reference materials would increase the total uncertainty of the analytical results. (b) Calibration introducing Hg in the gaseous phase. If this strategy is selected, standard addition or isotope dilution could serve to correct the matrix effects. The latter approach was considered the most promising one and, thus, further evaluated.

**Results Obtained with the Use of a Hg Generator.** *Calibration of the Hg Emitted by the Permeation Tube.* A permeation tube emitting isotopically enriched Hg was produced in-house for an accurate calibration of the mercury species. This simple device was described earlier in Procedure for the Generation of the Enriched Hg Spike for Isotope Dilution and is shown in Figure 1.

To determine the exact isotopic composition of the spike, all stable isotopes of Hg, $^{195}Pt$ (to correct for the spectral interferences of $^{196}Pt$ on $^{196}Hg$ and of $^{198}Pt$ on $^{198}Hg$) and $^{208}Pb$ (to correct for the spectral interference of $^{204}Pb$ on $^{204}Hg$) were measured by means of ICPMS. Corrections for dead time and mass discrimina-

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Table 3. Isotope Ratio and Emission Rate of Mercury of the In-House-Made Permeation Tube at 20.0 ± 0.1 °C

| | | AAS | AFS |
|---|---|---|---|
| isotope ratio $^{200}Hg/^{202}Hg$ | 11.34 | | |
| stability over 1 month | $s = 0.22$ | | |
| emission rate (ng $min^{-1}$) | | 0.379 | 0.386 |
| stability over 2 months | | $s = 0.026$ | $s = 0.020$ |
| $n$ | | 15 | 17 |

Table 4. Results Obtained with Single Standard Addition for TORT-2 ($\mu$g $g^{-1}$) ($n = 20$)

| TORT-2 | total Hg | methylmercury (as Hg) | inorganic Hg |
|---|---|---|---|
| certified value | 0.27 ± 0.06 | 0.152 ± 0.013 | (0.122)[b] |
| obtained by standard addition | 0.495<br>$s = 0.111$<br>RSD = 22% | 0.164<br>$s = 0.026$<br>RSD = 16% | 0.337<br>$s = 0.125$<br>RSD = 37% |

[b] total Hg − methyl Hg = inorganic Hg.

Table 5. Results Obtained with Isotope Dilution for TORT-2 ($\mu$g $g^{-1}$)[a]

| day | $n$ | total Hg | methylmercury |
|---|---|---|---|
| 1 | 17 | 0.274<br>RSD = 13.6% | 0.148<br>RSD = 8.4% |
| 2 | 25 | 0.324<br>RSD = 11.1% | 0.151<br>RSD = 8.2% |
| 3 | 20 | 0.297<br>RSD = 8.7% | 0.155<br>RSD = 6.8% |
| 4 | 20 | 0.237<br>RSD = 7.1% | 0.173<br>RSD = 4.7% |
| mean | | 0.286 | 0.157 |
| $s$ | | 0.037 | 0.011 |
| RSD (%) | | 13.0 | 7.2 |
| | | Total Hg (result ± 95% c.i.) | Methylmercury (result ± 95% c.i.) |
| certified value | | 0.27 ± 0.06 | 0.152 ± 0.013 |
| this work | | 0.286 ± 0.059 | 0.157 ± 0.018 |

[a] The determinations were carried out on four different days and the results of every session are presented.

tion were performed.[41] To create exactly the same conditions as for the sample measurements, the gaseous spike was also led through the graphite furnace first, where it underwent the temperature program. This is advisable, since a change in temperature may produce a shift in the zone of maximum $M^+$ density in the ICP and, thus, may have an influence on the signal (extraction efficiency).[42] The value finally obtained is presented in Table 3.

To determine the Hg emission rate, the Hg released from the permeation tube was collected during a given time interval on a gold-coated silica absorber (made in-house, consisting of a quartz glass tube, 2 plugs of quartz wool, and 30 mg of gold-coated silica (Phase Separations Ltd, U.K.)). The mercury on the absorber was released by thermal desorption and led into an atomic absorption spectrometer and an atomic fluorescence spectrometer, which were coupled on-line. Calibration of the spectrometers was performed by injecting a known amount of Hg-saturated air, obtained from a 350-mL closed flask containing 30 mL of Hg at a fixed temperature (±0.1 °C).[43] The results are presented in Table 3. As can be seen, there was a good agreement between the results obtained using the two methods.

*Results of the Analysis of TORT-2 and Analytical Figures of Merit.* Once the exact isotopic content of the spike and the emission rate were determined, the suitability of the gaseous spike for calibration of the solid sample was tested. The possibilities of single-standard addition (direct comparison of the signal from the sample with that of the sample plus the spike) were checked first. Samples of ∼1 mg were selected for the measurements. The results are presented in Table 4. As can be observed, although good results were obtained for methylmercury, the method cannot appropriately correct for the varying matrix effects and, thus, quantify the more problematic second peak (inorganic Hg), leading also to imprecise and inaccurate results for total Hg. The use of lower sample masses did not result in a significant improvement.

Isotope dilution is known to be the most effective way of correcting for changes in intensity by matrix effects, signal drift, instrument instability, and variations in the transport efficiency. An important advantage of this method when compared to standard addition is that isotope dilution can provide accurate results even if the matrix-induced signal suppression is not a reproducible effect. Rottmann and Heumann demonstrated the advantage of applying the isotope dilution technique in speciation studies, even when a species-unspecific spike is used.[44] Moreover, isotope dilution has also been successfully used in a previous work for the determination of volatile elements (Cd and Se) by solid sampling-ETV-ICPMS.[41]

Therefore, the capabilities of isotope dilution for the analysis of the TORT-2 were tested. Samples of ∼1 mg were selected for the determinations. For the calculation of the results, the approach proposed by Rottmann and Heumann was used.[44] In this method, the signals measured for each isotope are ratioed point by point. These ratios can be converted into the flow of detected Hg (pg $s^{-1}$) via the isotope dilution formula, as described in ref 44, and finally, integration of the two peaks can be carried out. Figure 5 illustrates this process, also proving that the method allows for an efficient correction of matrix effects. The final Hg flow vs time profile (Figure 5c) shows two well-defined peaks, in contrast with the signal suppression that can be observed in Figure 5a.

The results obtained are compiled in Table 5. Determinations were carried out on four different days (∼20 determinations every day), to test the robustness of the method; 10−15 determinations represent 1 h of work. As can be seen, the results were very satisfactory, because they showed a remarkable agreement with the certified values. The RSD values for 20 replicates were found to be between 5 and 14%. These values are acceptable considering the low amount of sample introduced into the vaporizer and can be mainly attributed to sample nonhomogeneity.[45]

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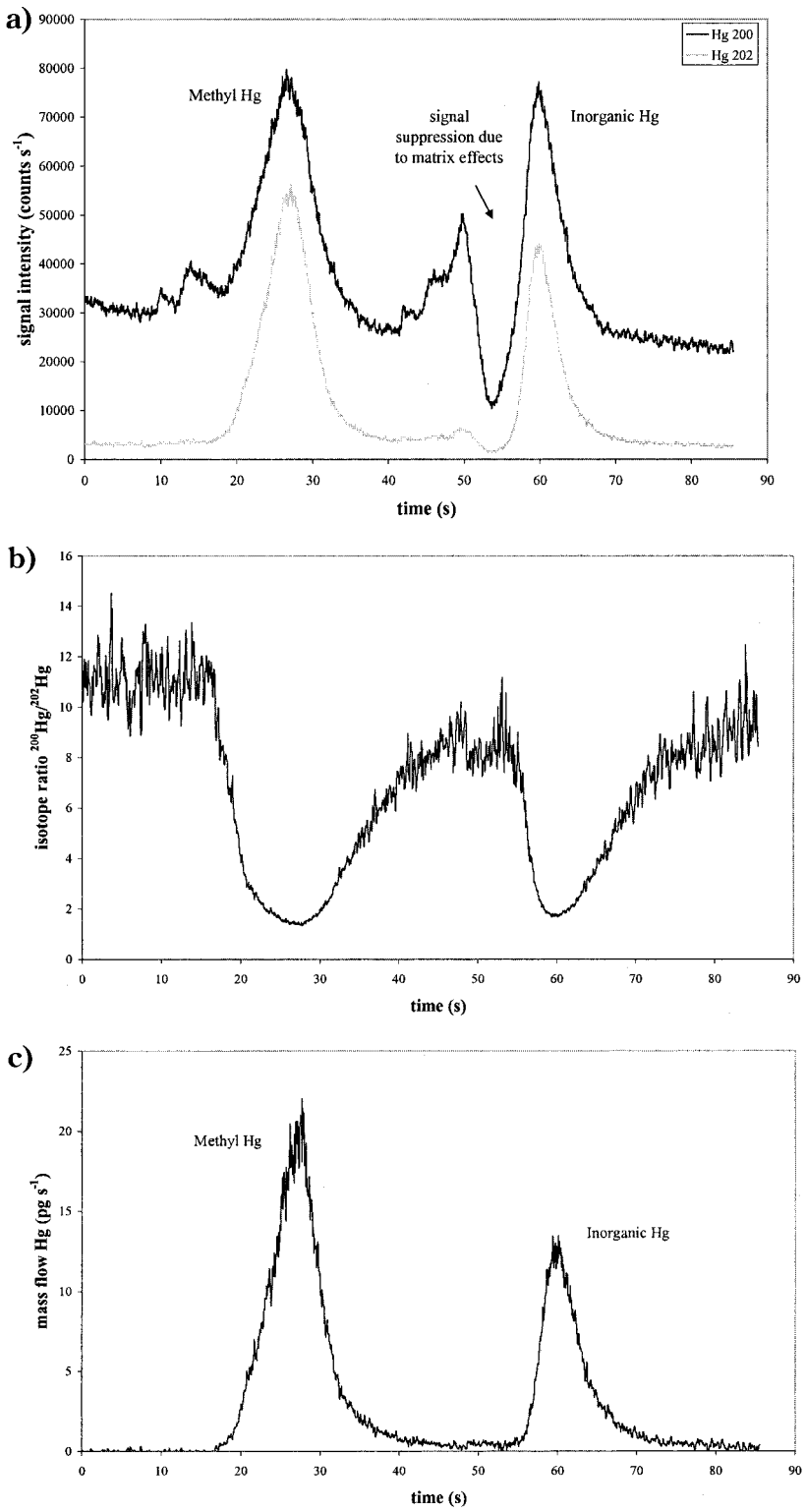


Figure 5. Signal profiles corresponding to (a) a solid sample (TORT-2; 0.992 mg) plus the spike, (b) the calculated $^{200}Hg/^{202}Hg$ isotope ratio, and (c) the calculated Hg mass flow (after integration, the first peak corresponds to 156 pg of Hg and the second one, to 91 pg of Hg).

The robustness of the ID approach can be further judged from Figure 6. No significant difference can be seen between results from different sample masses vaporized (between 0.2 and 1.2 mg), which is consequent with the appropriate correction of the matrix effects.

Finally, the detection limits obtained in this work, calculated as $3\sigma$ on 10 successive measurements of the blank, were compared with those obtained by other analytical methods (see Table 6).[2−6,8−15,22,46−48] Assuming that the contents in lobster hepatopan-

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(46) Liaw, M. J.; Jiang, S. J.; Li, Y. C. *Spectrochim. Acta B* **1997**, *52*, 779−785.
(47) Río-Segade, S.; Bendicho, C. *Spectrochim. Acta B* **1999**, *54*, 1129−1139.
(48) Jimenez, M. S.; Sturgeon, R. E. *J. Anal. At. Spectrom.* **1997**, *12*, 597−601.

Table 6. Detection Limits for Different Analytical Methods for Fish Samples

| analytical technique | total Hg, ng $g^{-1}$ | methylmercury, ng $g^{-1}$ | inorganic Hg, ng $g^{-1}$ |
|---|---|---|---|
| this work | | 2 | 6 |
| USS-ETV-ICPMS[46] | 2−4 | | |
| ETV-ICPMS, room temp. treatment with TMAH[22] | 50 | | |
| FI-(CV)AAS with on-line oxidation[47] | 32−74 | | 74 |
| GC−AAS, ethylation with $NaBEt_4$ [2] | | 120 | |
| GC−(QF)AAS, $NaBEt_4$ derivatization[3] | | 4 | 75 |
| HG-CT-GC−ETAAS, alkaline digestion[4] | | 3 | 3 |
| GC−AFS, one stage gold amalgamation (total Hg); $NaBEt_4$ (MeHg)[5] | 1 | 1.4 | |
| cryoGC−AFS, $NaBEt_4$ (MeHg), 2 stages gold amalgamation (total Hg)[6] | 0.5 | 0.1 | |
| isothermal multicapillary GC−AES after extraction[8] | | 20 | 80 |
| GC−(MIP)AES, derivatization $NaBEt_4$ [9] | | 16 | |
| GC−(MIP)AES in situ derivatization $NaBEt_4$ and nonane[10] | | 4.4 | |
| GC−FAPES, TMAH, $NaBEt_4$ [48] | | 1.4 | 0.2 |
| GC-ECD[11] | | 5 | |
| GC−ICPMS[10] | | 2.6 | |
| multicapillary GC−ICPMS[12] | | 0.2 | |
| HPLC−AFS, redox interface[13] | | 0.1 | |
| HPLC−postcolumn oxidation-(CV)AFS[14] | | 10 | |
| HPLC−HPF/HHPN-ICPMS[15] | | 0.025 | |

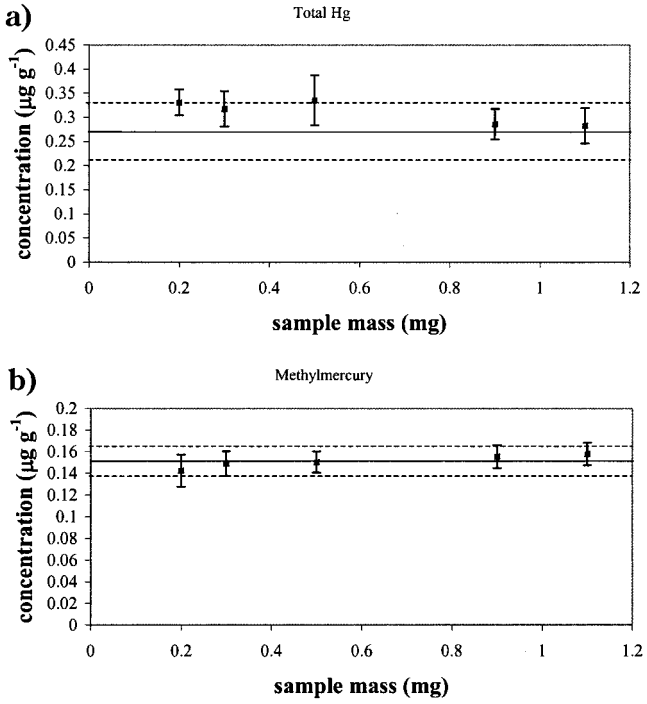


Figure 6. Results obtained with different sample masses (TORT-2) for (a) total Hg and (b) methylmercury.

creas are representative of the normal Hg contents in fish samples, it is clear that the sensitivity of this method is more than sufficient for this kind of application. It should be mentioned that the use of more modern ICPMS instrumentation could contribute to a decrease in these detection limits.

*Expansion of the Dynamic Range.* For the application of the method to other samples with a different analyte content (normally higher), the emission rate of Hg from the permeation tube must be adapted to the actual Hg content of the sample to keep the sample/spike ratio between the values usually recommended (0.1 and 10).[49] This can be simply achieved by changing the permeation tube temperature.

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Table 7. Parameters Used for Methylmercury Determination in DORM-2 and BCR CRM 463 and Results Obtained[a]

| parameter | | |
|---|---|---|
| temp program | same as for TORT-2 | |
| emission rate (32 °C) | 1.418 ng $min^{-1}$ | |
| stability over 1 month | $s = 0.032$ (RSD = 2.3%) | |
| isotope ratio $^{200}Hg/^{202}Hg$ | 11.58 | |
| stability over 1 month | $s = 0.24$ (RSD = 2.1%) | |
| results ± 95% c.i. ($\mu$g $g^{-1}$) | | |
| methylmercury | DORM-2 | BCR CRM 463 |
| certified | 4.47 ± 0.32 | 2.83 ± 0.16 |
| this work | 3.99 ± 0.61 | 2.47 ± 0.13 |

[a] $n = 5$ for DORM-2, and $n = 8$ for BCR CRM 463.

When the emission rate at a certain temperature ($T_0$) is known, the emission rate at a different temperature ($T$) can be calculated from the following equation,[50]

$$\log P = \log P_0 + \alpha(T - T_0)$$

where $P_0$ is the emission rate at temperature $T_0$; $P$ is the emission rate at temperature $T$, and $\alpha$ is the temperature coefficient characteristic for the permeation tube.

For commercially available permeation tubes, the temperature coefficient varies between 0.030 and 0.034 $°C^{-1}$.[50] For our in-house-built permeation tube, the emission rate was determined at different temperatures by AAS and AFS (see section 3.3.1). Plotting the log $P$ versus the temperature resulted in a linear curve ($\log P$ [ng $min^{-1}$] = 0.0458T [°C] − 1.3183, $r^2 = 0.9998$), from which the emission rate can be calculated at any temperature.

To carry out the analysis of two other reference materials (DORM-2, dogfish muscle and BCR CRM 463, tuna fish) in which Hg is present as methylmercury at a concentration level more than 10 times higher than in TORT-2, the temperature of the permeation tube was raised to 32 °C. Table 7 shows that both the emission rate and the measured isotope ratio are constant within

(50) http://www.vici.com/support/tn/tn1001.pdf.

2% over the entire test period of 1 month. As can be seen, the results obtained also showed a good agreement with the certified values.

## CONCLUSIONS

In this work, ETV-ICPMS has been successfully used for the fast and accurate direct determination of methylmercury and inorganic mercury in three different solid samples of marine origin (biological reference materials).

The method takes advantage of the possibilities of electrothermal vaporization to selectively vaporize the two mercury species in a single tube firing. To overcome the matrix effects resulting from the simultaneous vaporization of inorganic mercury and most of the organic matrix, species-unspecific isotope dilution has proven to be a very reliable calibration approach. A gaseous $^{200}$Hg-enriched spike could be obtained by using a simple in-house-built permeation tube.

The resulting method combines very interesting features, the most important being a high sample throughput (∼1 h/10−15 determinations), low detection limits (2 ng $g^{-1}$ and 6 ng $g^{-1}$ for methylmercury and inorganic mercury, respectively), low sample consumption (∼1 mg/measurement), low risk of sample contamination andr analyte losses (which can be important considering the high volatility of methylmercury species), and as a result of the lack of any sample pretreatment, the likelihood that the original chemical form of the analytes in the samples is preserved. Further research will be devoted to the application of the developed method to the routine analysis of real fish samples and also to check the applicability of the method to other materials, such as different biological samples, sediments, and sludges, which might exhibit a different vaporization behavior.

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