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Synthesis and characterization of isotopically enriched methylmercury ($CH_3{}^{201}Hg^+$)

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A simple procedure for the synthesis of an important standard, isotopically enriched methylmercury, which is not commercially available, has been established successfully. The isotopically enriched standard synthesized is utilized in conventional isotope dilution mass spectrometry (IDMS), as well as in speciated IDMS (SIDMS), for determination of the true concentration of methylmercury in environmental samples. The $CH_3{}^{201}Hg^+$ standard has been synthesized from commercially available ^{201}HgO and tetramethyltin. The synthesis time required is 1 h at 60 °C. The product is highly pure, yielding more than 90% as ^{201}Hg in $CH_3{}^{201}Hg^+$. Hazardous dimethylmercury does not occur during this synthesis procedure. The product synthesized was analyzed using high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (ICP-MS) and ICP-MS alone in order to determine its concentration, isotopic composition and purity. The stability of the product was also evaluated for over 6 months and found to be stable at 4 °C in the dark. The isotopically enriched methylmercury synthesized can be used in SIDMS and IDMS analyses as a standard.

KEYWORDS: isotopically enriched; methylmercury; ^{201}HgO; $CH_3{}^{201}Hg^+$; isotope dilution mass spectrometry (IDMS); speciated isotope dilution mass spectrometry (SIDMS)

INTRODUCTION

Over the past decades, the interest in speciation analysis has increased significantly due to the growing awareness that many organometallic compounds are more toxic than their corresponding free metals.[1] This is reflected in the increasing number of published papers based on the survey of *Analytical Abstracts* for the subject 'speciation' or 'species' since 1980. The number of papers published was relatively constant from 1981 to 1990, at an average of 75 papers per year. It then increased significantly from 118 papers in 1991 to 304 in 2002, at an average of 245 papers per year. Mercury is one of the most dangerous contaminants in the environment due to its accumulation in aquatic organisms and the phenomenon of 'bioamplification' through the trophic chain. The determination of total mercury is frequently not sufficient for understanding the toxicological impact and pathway of mercury species in the environment. The toxicity, bioaccumulation and environmental mobility of mercury are highly dependent on its chemical forms. The organometallic forms, especially methylmercury, are considered more toxic than inorganic mercury compounds because of their high affinity for thiol groups.[2] Environmental methylmercury originates largely from the methylation of inorganic mercury; major non-commercial sources of inorganic mercury are degassing of the Earth's crust, emissions from volcanoes, and evaporation from natural bodies of water.[3] One large anthropogenic source of inorganic mercury is the thermal conversion and volatilization of mercury compounds in coal used worldwide in large quantities in unremediated coal-fired power plants. Anthropogenic emission of methylmercury can be produced by biological activity on inorganic mercury in bottom sediments, decomposed fish and biological activity in soil.[4,5] Methylmercury formed in these ways is introduced into the food chain and humans ingest it mainly through diet. The main target of methylmercury in humans is the central nervous system—especially the sensory, visual and auditory areas involved in coordination. The most severe effects lead to widespread brain damage, resulting in mental derangement,

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coma, and death.[6] Therefore, it is essential to determine the exact concentration of inorganic mercury and methylmercury present in environmental, biological and food samples.

Most of the published methods for mercury speciation in environmental samples are based on the Westöö procedure[7] (an acid leaching method), solvent extraction,[8–11] distillation,[8,12,13] or modification of Westöö methodology[14] (alkaline-based leaching) and supercritical fluid extraction.[15] The most widely used separation techniques are: gas chromatography (GC), high-performance liquid chromatography (HPLC) coupled with element-selective detection techniques such as atomic emission spectrometry (AES), atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS) or cold vapor AAS (CV-AAS). As all of the extraction methods use either acid or base with organic solvents, and after extraction most of them go through some kind of preconcentration steps (e.g. ethylation or reduction with $SnCl_2$, or hydride generation with $NaBH_4$), there is a possibility of interconversion or unidirectional transformation of inorganic mercury to organic mercury or *vice versa* during the sample storage, shipment, extraction, preconcentration or analysis steps. Therefore, the results obtained using these procedures frequently introduce biases for either inorganic mercury or methylmercury, or both. In the literature, it was found that some of the researchers used isotope dilution mass spectrometry (IDMS) to determine the concentration of methylmercury from environmental samples by labeling methylmercury with isotopically enriched methylmercury.[16–19] By using this technique, it is possible to determine the amount of methylmercury present in sample during extraction but the data do not reveal anything about the source of methylmercury, i.e. whether this methylmercury is from the sample or is a product of methylation of inorganic mercury during extraction, preconcentration and/or analysis. In order to obtain true results from the extraction or analysis of environmental samples, it is required to label both the methylmercury and inorganic mercury with isotopically enriched methylmercury and inorganic mercury. This can be achieved by using EPA Method 6800 (*Elemental and Speciated Isotope Dilution Mass Spectrometry, SIDMS*).[20] SIDMS maintains the advantages of IDMS while facilitating the tracing of the species conversions after spiking and providing the ability to make corrections. In SIDMS, each species is 'labeled' with a different isotopically enriched spike in the corresponding species form; therefore, the interconversion and degradation that occur after spiking are traceable and can be corrected.[21,22] However, in spite of the benefits of SIDMS, it is not being used widely as a method of analysis because of the commercial absence of isotopically enriched methylmercury. According to the US EPA,[23] Method 6800 'is currently the only available means to make accurate and defensible speciated measurements [and] will serve as the reference method to define the species present in waste and environmental samples'.

According to a literature survey, there are some proposed methods for the production of organomercury compounds, e.g. the reaction of tetramethyltin ($(CH_3)_4Sn$) with inorganic mercury,[16,24] the reaction between inorganic mercury and dimethylmercury,[25] and the reaction of methylcobalamin (MeCo, a vitamin B_{12} analog) with inorganic mercury.[26–30] In most cases, dimethylmercury was produced along with monomethylmercury in the first step and then the dimethylmercury was converted to monomethylmercury. The production of dimethylmercury depends mainly on the reaction time, temperature and the ratio of inorganic mercury to methylcobalamin used. The principal focus of most of these studies[24,25,28–30] was the reaction product of the $(CH_3)_4Sn$ or methylcobalamin with inorganic mercury, not the synthesis of methylmercury with high purity and higher yield in order to use it as a standard compound. Only a few studies[16,26,27] were for the synthesis of isotopically enriched methylmercury. Rouleau and Block[27] carried out the synthesis using inorganic $^{203}Hg(II)$ and methylcobalamin with single-step isolation with hexane/benzene (1 : 1) and the final solution was prepared into Na_2CO_3. The yield was 90% and time required was less than 4 h. Hintelmann and Evans[16] carried out the synthesis by reacting inorganic $^{201}Hg(II)$ and tetramethyltin with six steps of extraction and purification: (i) extraction with toluene; (ii) wash the extract with double deionized (DDI) water; (iii) extract into 1 mM $Na_2S_2O_3$; (iv) wash with toluene; (v) add $CuSO_4$ and NaCl into the $Na_2S_2O_3$ extract; (vi) final extraction of methylmercury in toluene. No data were available for the percentage yield; however, it was reported that the time required was less than 4 h to complete the procedure. In both of these methods the full reaction conditions were not provided. On the other hand, Martín-Doimeadios *et al*.[26] synthesized isotopically enriched monomethylmercury using inorganic $^{201}Hg(II)$ with methylcobalamin with single-step extraction and purification. The time required was reported as less than 2 h and yield was about 90%. This method studied several parameters: pH, temperature, reaction time, and methylcobalamin to inorganic mercury ratio. Some of the methods suffer from disadvantages, such as low yield (50–70%), long reaction time (1 day) and multi-step purification.

Therefore, the purpose of this study was to investigate and optimize the synthesis of isotopically enriched methylmercury by using inorganic $^{201}Hg(II)$ and $(CH_3)_4Sn$ as the starting material so as to achieve higher yield, shorter reaction time and fewer purification steps, and to evaluate the isotopic composition, purity and stability of the product over a practical shelf-life (e.g. 6 months) by using HPLC–ICP-MS.

EXPERIMENTAL

Instrumentation

A CostaMetric 4100Bio/MS polymeric inert pump (Thermo Separation Products, Riviera Beach, FL, USA) and a 5 μm

Supelcosil LC-18 HPLC column with a Pelliguard LC-18 guard column (Supelco, PA, USA) were used in this study to separate inorganic and methylmercury. A six-port injection valve (Valco Vicci) was used between the pump and column. Because no special interface is required between the LC-18 column and the ICP mass spectrometer, one outlet of the column is interfaced directly to the nebulizer of the ICP mass spectrometer with a piece of Teflon tubing, and the other end is connected to a 50 µl TEFZEL™ sample loop (CETAC Technologies, Omaha, NE). Figure 1 shows a typical separation of inorganic and methylmercury using this system at a flow rate of 1.0 ml min^{-1}. The mobile phase was buffered 30% methanol (refer to Reagents and Standards section).

An HP 4500 ICP-MS (Agilent Technologies, USA, and Yokogawa Analytical System Inc., Japan) was used in this study. The sample delivery system consisted of a peristaltic pump and quartz spray chamber with concentric nebulizer and quartz torch. The instrument was fitted with platinum sampler and skimmer cones and was optimized daily using 10 ppb tuning solution (Agilent Technologies, USA) containing lithium, yttrium, cerium and thallium in 30% methanol. Time-resolved analysis (TRA) mode was engaged. The operating conditions for the LC–ICP-MS set-up are given in Table 1.

A direct mercury analyzer (DMA-80, Milestone GmbH, Germany) was used in this study to determine the total mercury content in each of the extraction and purification steps. The operation conditions for the DMA-80 used throughout this work were based on the guidelines provided in EPA Method 7473 protocol.[31,32]

Table 1. HPLC–ICP-MS operating conditions

Plasma	
Plasma flow rate (l min^{-1})	15.0
Auxiliary gas flow rate (l min^{-1})	1.0
Radio-frequency power (W)	1450
Sample cone	Platinum, 1.1 mm orifice
Skimmer cone	Platinum, 0.89 mm orifice
Measurement parameters	
Analysis mode	TRA
Analysis isotopes[a]	^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg
Nebulizer gas flow rate (l min^{-1})	0.93–1.00
Peristaltic pump rate (rpm)	0.25
Integration time per point (s)	0.5
Total analysis time (s)	400
Eluent flow rate (ml min^{-1})	1.0

[a] The isotope ^{204}Hg was not analyzed because of interference from ^{204}Pb.

Reagents and standards

DDI water (18 MΩ cm^{-1}), prepared from a Barnstead NANO pure Ultrapure Water System (Dubuque, Iowa, USA), was used in the preparation of all solutions throughout this study. Reagent-grade HCl, Na_2SO_4, $Na_2S_2O_3$, toluene, isopropanol, ammonium acetate, 2-mercaptoethanol (98%), and optima-grade methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). The reagent-grade tetramethyltin (98%) was obtained from Alfa Aesar (Ward Hill, MA, USA).

Standard solutions containing 1 mg ml^{-1} of $HgCl_2$ in 5% HNO_3 and CH_3HgCl in water were commercially available from Alfa Aesar (Ward Hill, MA, USA). ^{201}HgO, Lot # VX3060, was obtained from Isotech Inc. (Miamisburg, OH, USA). The natural and enriched isotope abundance of mercury standards are listed in Table 2.

HPLC speciation mobile phase (30% (v/v) methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate), modified from Wilken's procedure,[33] was prepared by diluting 300 ml of methanol, 50 µl of 2-mercaptoethanol and 4.8 g of ammonium acetate in 700 ml of DDI water.

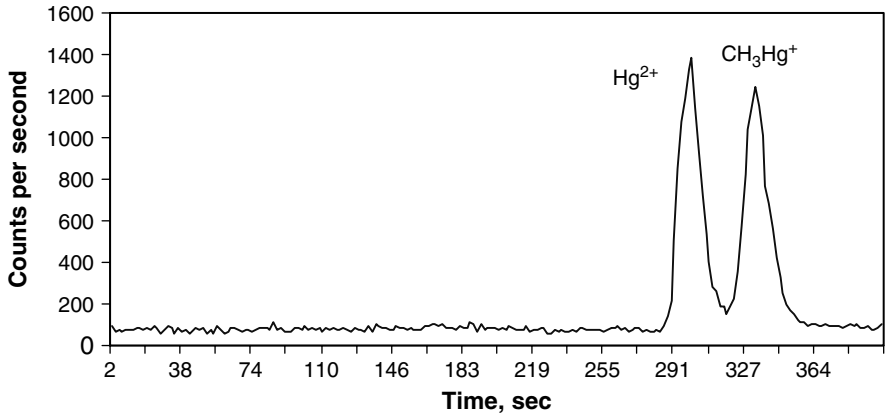

Figure 1. Typical chromatogram for separation of inorganic mercury and methylmercury. Flow rate: 1 ml min^{-1}; eluent: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate; column: 5 µm Supelcosil LC-18 HPLC column.

Table 2. Results for characterization of natural abundance and synthesized isotopically enriched methylmercury with ICP-MS

	Natural abundance		Enriched ^{201}HgO		Enriched $CH_3{}^{201}Hg^+$
Mass	Reported	Determined	Certified	Determined	determined
196	0.15	0.179 ± 0.020	<0.05	0.012 ± 0.001	0.025 ± 0.004
198	9.97	10.049 ± 0.035	0.08	0.108 ± 0.033	0.132 ± 0.040
199	16.87	16.966 ± 0.034	0.10	0.155 ± 0.061	0.200 ± 0.080
200	23.10	23.049 ± 0.106	0.45	0.637 ± 0.096	0.658 ± 0.094
201	13.18	13.381 ± 0.205	98.11	97.707 ± 0.316	97.530 ± 0.352
202	29.86	29.569 ± 0.078	1.18	1.270 ± 0.100	1.316 ± 0.117
204	6.87	6.809 ± 0.027	0.08	0.111 ± 0.027	0.139 ± 0.026
Total	100.00	100.000 ± 0.251	100.00	100.000 ± 0.353	100.000 ± 0.394

Procedure

Synthesis of ^{201}Hg-enriched methylmercury

In order to prepare $^{201}HgCl_2$, 6 ml of $^{201}Hg^{2+}$ solution (11 μg ml^{-1}) was mixed with 2 ml of 6.0 M HCl in a 20 ml amber glass vial and stirred for 5 min. A 0.93 M methanolic solution of $(CH_3)_4Sn$ was prepared by mixing 0.340 g of $(CH_3)_4Sn$ into 2 ml methanol and then the mixture was transferred quantitatively into the $^{201}HgCl_2$ solution and the glass vial cap was put back on. The resulting reaction mixture was then stirred for 1 h at 60 °C in a water bath. The reaction mixture was cooled to room temperature and extracted three times with toluene (4 + 3 + 3 ml).

Purification procedure

The methylmercury synthesized (in toluene) was then washed with DDI water three times (4 + 3 + 3 ml). 2.5 ml of the toluene extract was then dried over Na_2SO_4 and diluted with isopropanol (1 : 1, v/v). Another 2.0 ml of the toluene extract was taken and extracted twice with 2.5 ml of 1% $Na_2S_2O_3$. All of the extracts were stored in amber glass vials in a cold room at 4 °C until analysis.

Availability of isotopically tagged methylmercury

To assist in the use of SIDMS, some isotopically tagged species will be provided to academic research upon request from this research group at Duquesne University[34] and will be available as a commercial product from Applied Isotope Technologies (e-mail: appliedisotopes@comcast.net).

RESULTS AND DISCUSSION

Optimization of synthesis conditions

A total of five methylmercury syntheses were performed during this study. The Hintelmann and Evans[16] procedure for synthesis and purification of isotopically enriched methylmercury was followed step by step at the beginning of this study. The preliminary study was done using natural abundance HgO and $(CH_3)_4Sn$. The effect of HCl concentration, temperature, reaction time, inorganic mercury to $(CH_3)_4Sn$ ratio, and number of purification steps required were studied. Mercury present in the reaction mixture (left after toluene extraction), in the water wash, in the first toluene extract, in the toluene wash, in the 1% $Na_2S_2O_3$ extract, in the NaCl + $CuSO_4$ fraction, and in the final toluene extract were all analyzed as total mercury using the DMA-80. Only the methylmercury present in the first toluene extract, in the 1% $Na_2S_2O_3$ extract and in the final toluene extract from preliminary studies were analyzed by HPLC–ICP-MS. The results from the DMA-80 and HPLC–ICP-MS analyses agree with each other. The final results and the respective synthesis conditions are reported in Table 3. The results are presented as percentage recovery in parentheses and mercury content in each fraction in microgram units.

From Table 3, it is found that the percentage yield increased from 47.9% (synthesis 1) to 67.9% (synthesis 2) with the increase of the HCl concentration from 0.1 to 6.0 M. Therefore, 6.0 M HCl was used during the rest of the study. The percentage yield increased from 67.9% (synthesis 2) to 92% (synthesis 3) by increasing the temperature from 20 °C (room temperature) to 60 °C. Therefore, the final synthesis was performed at 60 °C. By studying the reaction time it was found that the percentage yield does not depend significantly on reaction time. Therefore, 1 h was selected for the final synthesis procedure. From Table 3, it was also found that the ratio of inorganic mercury to $(CH_3)_4Sn$ has no effect on percentage yield.

During HPLC–ICP-MS analysis of the first toluene extract only methylmercury was detected; no unreacted inorganic mercury or dimethylmercury was found (Fig. 2). Also from the data in Table 3, it is found that the percentage yield of methylmercury does not change significantly from the first toluene extract to the final toluene extract. In all of the cases, the values were less than 4%. However, there are three steps between the first toluene extract and the final toluene extract. It was decided to purify the methylmercury synthesized by washing the first toluene extract with DDI water and then drying over Na_2SO_4, then diluting with isopropanol to prepare the working standard. Unfortunately, during application of the isotopically enriched

Table 3. Results for the preliminary and final synthesis of isotopically enriched methylmercury. Analysis using the DMA-80 and HPLC–ICP-MS[a]

	Mercury content (μg) [recovery (%)]				
Synthesis step	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Reaction mixture	5990 [40.4]	5168 [31.0]	379 [2.9]	3.4 [3.6]	2.5 [3.8]
Water wash	791 [5.3]	34 [0.2]	275 [2.1]	0.2 [0.2]	1.5 [2.3]
First toluene extract	8031 [54.2]	11 470 [68.8]	12 355 [94.6]	91.2 [96.0]	61.8 [93.7]
Toluene wash	139 [0.9]	85 [0.5]	157 [1.2]	3.3 [3.5]	0.5 [0.8]
$Na_2S_2O_3$ extract	7885 [53.2]	11 350 [68.1]	12 130 [92.9]	87.8 [92.4]	61.2 [92.7]
$NaCl + CuSO_4$ fraction	768 [5.2]	10 [0.1]	15 [0.1]	1.1 [1.2]	–
Final toluene extract	7105 [47.9]	11 325 [67.9]	12 010 [92.0]	86 [90.5]	–
Total	14 793 [99.8]	16 622 [99.7]	12 836 [98.3]	94 [98.9]	65.7 [99.6]

[a] *Synthesis conditions*. Trial 1: 16 mg HgO, 2 ml 0.1 M HCl, 5 min, 0.385 g $(CH_3)_4Sn$, 3 h, room temperature. Trial 2: 18.0 mg HgO, 2 ml 6.0 M HCl, 5 min, 0.385 g $(CH_3)_4Sn$, 3 h, room temperature. Trial 3: 14.1 mg HgO, 2 ml 6.0 M HCl, 5 min, 0.385 g $(CH_3)_4Sn$, 3 h, 60 °C. Trial 4: 95 μg $^{201}Hg^{2+}$, 2 ml 6.0 M HCl, 5 min, 0.385 g $(CH_3)_4Sn$, 3 h, 60 °C. Trial 5: 66 μg $^{201}Hg^{2+}$, 2 ml 6.0 M HCl, 5 min, 0.340 g $(CH_3)_4Sn$, 1 h, 60 °C.

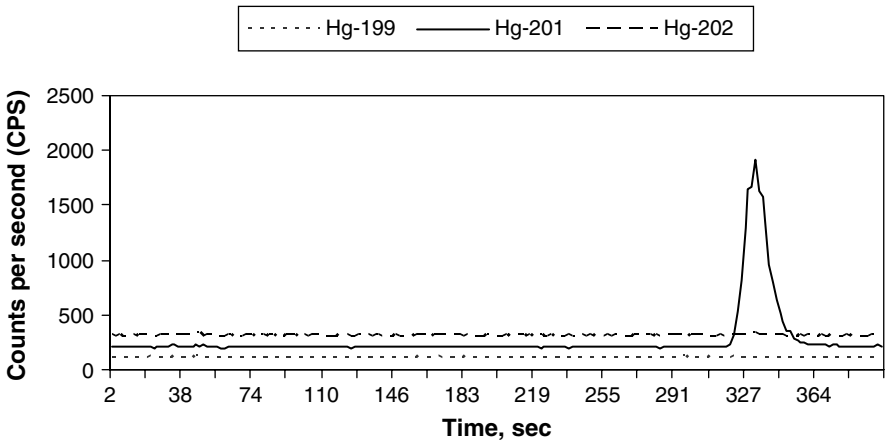

Figure 2. Chromatogram for synthesized isotope-enriched methylmercury ($CH_3{}^{201}Hg^+$). Chromatograms for different masses (^{202}Hg, ^{201}Hg and ^{199}Hg) were shifted from the baseline by adding 300 CPS, 200 CPS and 100 CPS respectively to the original counts for clarity. Flow rate: 1 ml min^{-1}; eluent: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate; column: 5 μm Supelcosil LC-18 HPLC column.

methylmercury synthesized (in isopropanol or in the toluene extract) in SIDMS analysis, it was found that the product synthesized induced both the sample inorganic mercury and the isotope-enriched $^{199}Hg^{2+}$ to convert to methylmercury. The chromatogram shown in Fig. 3 was obtained from a blank analysis with HPLC–ICP-MS. The blank was prepared by spiking equal amounts of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in DDI water and keeping it on the bench top at room temperature for 6 h. This chromatogram shows that inorganic mercury has converted to methylmercury more than 90% within 6 h of equilibration without any treatment. Therefore, it was decided to include one more step in the purification procedure, i.e. washing the first toluene extract with DDI water, and then extracting it into 1% $Na_2S_2O_3$(aq.). A blank was then prepared by spiking $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in DDI water and keeping it on the bench top at room temperature for 6 h. The blank was then analyzed by HPLC–ICP-MS. No transformations between inorganic mercury and methylmercury were observed for $CH_3{}^{201}Hg^+$ extracted into 1% $Na_2S_2O_3$(aq.) (Fig. 4).

Characterization of the isotopically enriched methylmercury synthesized

After successful optimization of the synthesis procedure, an isotope-enriched methylmercury ($CH_3{}^{201}Hg^+$) was synthesized using ^{201}HgO and $(CH_3)_4Sn$ and analyzed using HPLC–ICP-MS (Fig. 2). The chromatogram contains no inorganic mercury nor any other mercury peaks but the methylmercury peak. In order to compare the peak position of the methylmercury synthesized with the naturally abundant methylmercury, these two standards were mixed at 1 : 10 ratio and analyzed by HPLC–ICP-MS (Fig. 5). This chromatogram

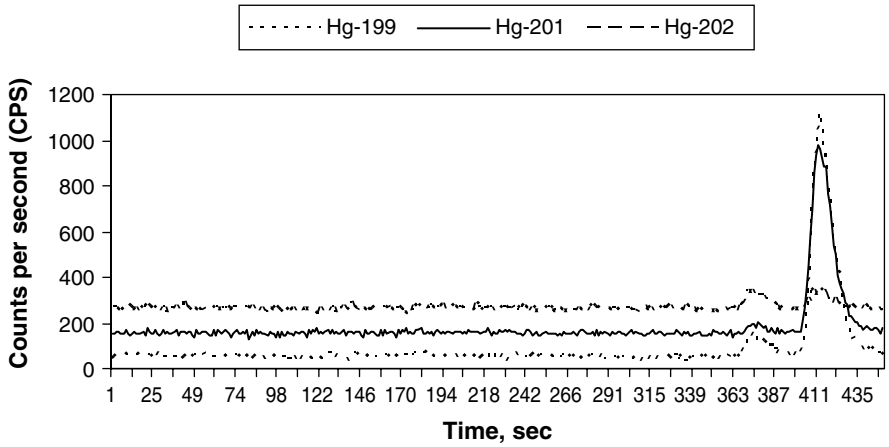

Figure 3. Chromatogram for a mixture of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in isopropanol. The mixture was kept on a bench top at room temperature for 6 h for equilibration. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 200 CPS and 100 CPS respectively to the original counts for clarity. Flow rate: 0.8 ml min^{-1}; eluent: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate; column: 5 μm Supelcosil LC-18 HPLC column.

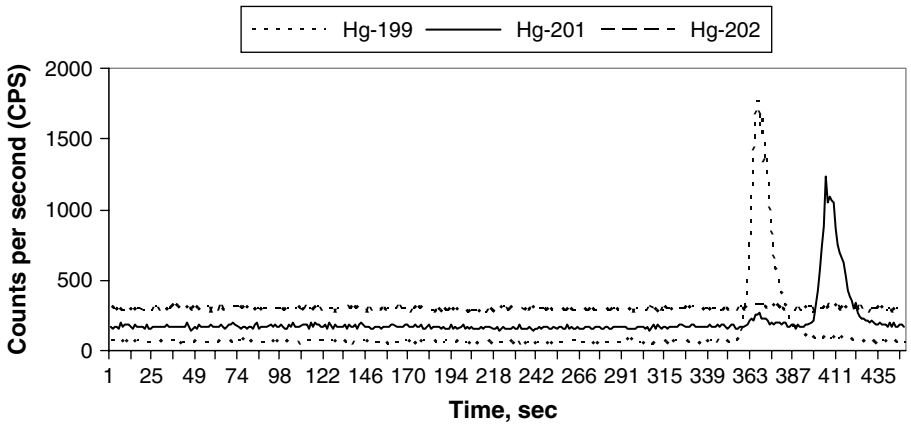

Figure 4. Chromatogram for a mixture of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in 1% $Na_2S_2O_3$. The mixture was kept on a bench top at room temperature for 6 h for equilibration. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 200 CPS and 100 CPS respectively to the original counts for clarity. Flow rate: 0.8 ml min^{-1}; eluent: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate; column: 5 μm Supelcosil LC-18 HPLC column.

shows that both preparations overlapped each other and appeared as a single peak at similar elution times, confirming that the product synthesized is the isotope-enriched methylmercury.

The isotopic abundances of the naturally abundant methylmercury (CH_3Hg^+) and the isotope-enriched ^{201}HgO were evaluated in order to compare the true measured isotope abundances with the reported natural abundance[35] and the isotope-supplier's certified value. This study was done using ICP-MS. The standard solutions were aspirated in direct mode and all isotope ratios were calculated for each species, and then the abundance of each isotope was calculated for each species. The results are reported in Table 2 with 95% confidence intervals. The values determined agree with the reported and certified values in most cases, and, as expected, the most enriched isotope in ^{201}HgO is ^{201}Hg compared with the natural abundance of methylmercury.

After synthesis of the isotopically enriched methylmercury its isotope abundances were also determined using the same procedure as described previously; these are also reported in Table 2 with 95% confidence levels. The values measured correspond well with the certified values in most cases.

The concentration of the isotopically enriched methylmercury synthesized in 1% $Na_2S_2O_3$ was determined by reverse IDMS (RIDMS) using two different approaches. First, the isotope-enriched methylmercury synthesized was mixed with naturally abundant methylmercury in 1 : 10 ratio and aspirated in direct mode to the ICP mass spectrometer five times

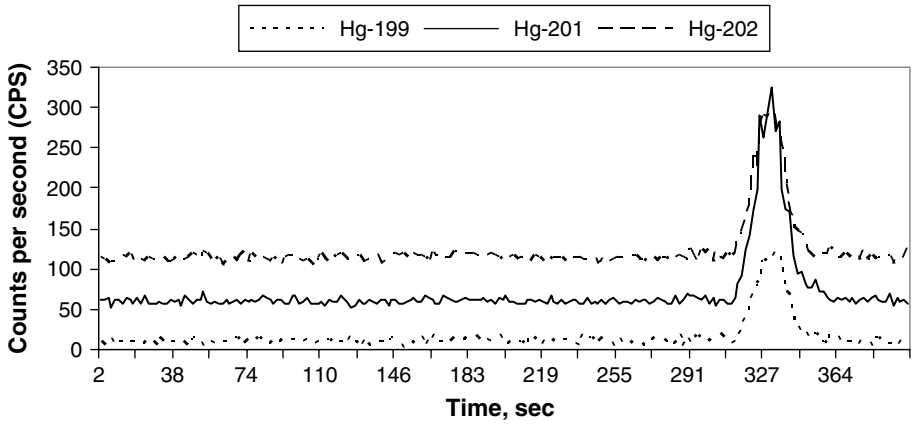

Figure 5. Chromatogram for a mixture of natural abundance and isotopically enriched methylmercury. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 100 CPS and 50 CPS respectively to the original counts for clarity. Flow rate: 1 ml min^{-1}; eluent: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate; column: 5 μm Supelcosil LC-18 HPLC column.

and measured in five replicates for each introduction. The isotope ratio of ^{201}Hg/^{202}Hg was determined with and without deadtime[36] and mass bias correction.[37] From the isotope ratios obtained, the concentration of $CH_3{}^{201}Hg^+$ was calculated using RIDMS and found to be 2.41 ± 0.01 μg g^{-1} and 2.52 ± 0.01 μg g^{-1} respectively. The concentration indicates the yield is 91.3 ± 0.4%. Second, the mixture of the isotopically enriched methylmercury synthesized and the naturally abundant methylmercury was analyzed using HPLC–ICP-MS four times. The isotope ratio of ^{201}Hg/^{202}Hg was determined with deadtime and mass bias correction, and the concentration of $CH_3{}^{201}Hg^+$ calculated using RIDMS and found to be 2.54 ± 0.21 μg g^{-1}. The concentration values obtained from both of these analyses correspond to each other at the 95% confidence level. Also, from HPLC–ICP-MS analysis, it was found that the product is 100% pure in methylmercury.

The concentration of the $CH_3{}^{201}Hg^+$ standard synthesized in 1% $Na_2S_2O_3$ was determined by RIDMS on 2 October 2002 as 2.41 ± 0.01 μg g^{-1}, on 10 November 2002 as 2.32 ± 0.23 μg g^{-1} and again on 30 March 2003 as 2.40 ± 0.01 μg g^{-1}. The concentrations of the standard synthesized over 180 days are not statistically distinguishable at the 95% confidence level. The concentration of the standard will continue to be checked for stability over time. The standard synthesized has successfully been used for the validation of proposed EPA Method 3200 (*Mercury species by selective solvent extraction and acid digestion*).

CONCLUSIONS

A highly pure isotopically enriched methylmercury, $CH_3{}^{201}Hg^+$, has been synthesized from commercially available ^{201}HgO and $(CH_3)_4Sn$ with a yield of more than 90% in a synthesis procedure lasting less than 1.5 h at 60 °C. This procedure increases the efficiency of the previous synthesis by ~1.8 times while providing for stability and purity. The synthesized and purified product is stable and does not induce transformation of the inorganic mercury to methylmercury during SIDMS or IDMS analysis of environmental samples. The health hazard of dimethylmercury is also eliminated during the synthesis procedure. This synthesis procedure is a safe and an environmentally green protocol. Isotopically tagged species are necessary for application of SIDMS and must be made or obtained to use this method. Some of these species are now available for use in speciated analysis.

Acknowledgements

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