Chapter

Studied the Methyl and Ethylmercury Artifacts in Biological Samples Using Sodium Tetra(n-Propyl)Borate as a Derivatizing Agent

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Abstract

Sodium tetra (n-propyl) borate was used as derivatizing agent to measure methyl and ethylmercury compounds. This study investigated the artifact formation of methyl and ethylmercury compounds during derivatization using $NaBPr_4$, simultaneously with the influence of this artifact on methylmercury analysis in biological samples (chlor alkali hair samples). The artifact methylmercury and ethylmercury compounds during derivatization using $NaBPr_4$ were evident and depended strongly on the amount of inorganic mercury (Hg^{2+}) present in the sample solution for derivatization and depended on the purity of sodium tetra (n-propyl) borate reagent. The high formation rate of artifact Et-Hg (0.76–0.81% of high-level Hg^{2+} present) interferes strongly with the ethylmercury analysis. The rate of artifact formation of Me-Hg is small and constant at the different concentration ranges of In-Hg (0.012% of In-Hg present) and does not affect on Me-Hg analysis and it can be subtracted from this Me-Hg artifact ratio from the measured value of Me-Hg in the biological samples. However, the mathematical correction for Me-Hg measurement can be done only when the Et-Hg peak is already appearing in the chromatogram samples.

Keywords: inorganic mercury, monomethyl ethylmercury, sodium tetra (n-propyl) borate, artifacts derivatization, mercury compounds

1. Introduction

Mercury has been well known as an environmental toxin and pollutant for several decades. The environmental cycling of mercury is a very complex distribution, involving a large variety of physical and chemical processes that affect its toxicity and mobility [1–4]. The lengthy mercury transport cycle in the atmosphere, it is deposition, bioaccumulation, and the concentration of extremely hazardous methylmerury

methylmercury (Me-Hg) molecules in the aquatic food chain represent a severe environmental concern, even in distant places, poisoning people [3–7].

Analytical techniques for the separation of methylmercury (Me-Hg) are well documented [8]. After extraction from solid matrices and derivatization, the methylmercury (Me-Hg) is frequently measured using hyphenated techniques [8]. Mercury speciation analysis is usually performed by resorting to hyphenated techniques, based on the coupling of an effective separation technique to a sensitive element-specific detector. Capillary gas chromatography (CGC), liquid chromatography (LC), or more recently capillary electrophoresis (EC) can be interfaced with specific atomic detection including atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), electron capture, or inductively coupled plasma mass spectrometry (ICPMS) [9–17]. Recently research has shown that the coupling of GC to ICP-MS appears to be a more suitable hyphenated technique to carry out the mercury speciation analysis because of its high sensitivity, multi-isotopic, and multi-elemental capabilities [8, 9, 15]. However, the GC-ICP-MS is only suitable for volatile species like mercury species [11, 15]. The isotope dilution ICP-MS is a new powerful approach and offers great potential for very small uncertainties since quantitative recoveries are not required and rearrangement reactions are easily detected [10, 17–21]. The main advantage of this technique (IDMS) is that chemical separation if required for accurate ratio determination need not be quantitative. Moreover, concentrations of chemical species can be measured very precisely because ratios can be measured very reproducibly [8, 11, 16].

Quality results are sometimes associated with sample pre-treatment; the analysis of solids such as biological and environmental samples requires leaching (alkaline or acid)/digestion step to liberate mercury species from the sample matrix before detection with GC-ICP-MS. However, for ionic mercury species, derivatization reactions are required to achieve good results [11, 22].

In earlier studies, monomethyl mercury (Me-Hg) was the most investigated organomercury compound, and measurement of monomethylmercury (Me-Hg) in environmental samples using sodium tetraethylborate ($NaBEt_4$) was one of the most used methods for methylmerury analysis [23, 24]. However, in some cases during the ethylation (Eth) with sodium tetraethyl borate ($NaBEt_4$), the Hg^{2+} is transformed to $HgEt_2$, while MeHg forms MeHgEt Eqs. (1) and (2).

$$Hg^{2+} + 2NaB\,(C_2H_5)_4 \rightarrow Hg\,(C_2H_5)_2 + 2Na^{+} + 2B(C_2H_5)_3 \quad (1)$$

$$CH_3Hg^{+} + NaB\,(C_2H_5)_4 \rightarrow CH_2HgC_2H_5 + Na^{+} + B(C_2H_5)_3 \quad (2)$$

As mentioned above, isotope dilution ICP-MS is a new powerful approach to solving the problems with the matrix and non-quantitative derivatization. A drawback of the ethylation (Eth) procedure is the impossibility to distinguish between Hg^{2+} and $EtHg^{+}$, both species that often coexist in the environment [25]. It was observed that derivatization using ethylation reagent ($NaBEt_4$) induced the formation of MeHg from inorganic mercury (InHg) if inorganic mercury was present at high concentrations and also the presence of dissolved organic matrix in the sample strongly interferes with ethylation process [18, 26, 27]. Therefore, ignoring this effect of artifact formation may lead to systematic errors in methylmercury analysis. Recently, an alternative is the use of the propylation as a derivatization technique with sodium tetra-propyl borate as the derivatizing agent which is more tolerant to interferences from chlorides [11, 18, 26, 28]. However, it was found that the artifact of methylmercury (Me-Hg) and ethylmercury (Et-Hg) compounds during $NaBPr_4$ derivatization

was evident and depended strongly on the concentration of inorganic mercury (Hg^{+2}) presence in the solution for derivatization. For example, Jen-How Huang [26] observed a transformation of In-Hg into ethylmercury (Et-Hg) and methylmerury (Me-Hg) during derivatization using $NaBPr_4$, and he reported that the artifact formation rates of EtHg and MeHg are 0.99–2.9% and 0.03–0.28%, respectively. This conclusion may ignore the artifact formation of monomethylmercury (Me-Hg) and monoethylmercury (Et-Hg) during derivatization by $NaBPr_4$ similar to $NaBEt_4$. Therefore, without taking this effect of artifact formation into account, the artifact may lead to an overestimation of organomercury species concentrations and a false impression of organomercury speciation.

This study aims to investigate the formation of Me-Hg and Et-Hg artifacts in hair samples with the high level of In-Hg in hair workers of ICL factory in Pakistan by comparing the Hg artifacts in un-spiked and spiked blank samples, different concentrations of normal abundance In-Hg solution, enriched ^{199}In-Hg solution and hair sample (normal hair) with low level (0.98 mg/kg) of In-Hg during the derivatization step.

The objectives are [1] to examine the artifact formation of methyl and ethylmercury from inorganic mercury (Hg^{2+}) during propylation using $NaBPr_4$, [2] to identify the factors which govern the artifact formation of MeHg and EtHg, and [3] to evaluate the influence of MeHg and EtHg artifact information on the determination of actual monomethylmercury (Me-Hg) concentrations in chlor alkali hair samples with high inorganic mercury concentrations (Up to 0.9%).

2. Materials and methods

2.1 Devices and instrument

Microwave digestion oven model MARS-5 from CEM Instrument, UK, was used for digestion and decomposition of hair samples. The microwave operating conditions are listed in **Table 1**. A gas chromatograph (GC) model HP 6850 outfitted with a capillary column was connected to an Agilent model HP-7500 ICP mass spectrometer through a heated steel transfer capillary for speciated isotope dilution analysis (SIDMS). The heated steel transfer capillary was inserted into the ICP torch injector, and connection to the torch was realized through a glass T-piece. A conventional Meinhard concentric nebulizer and low volume water-cooled cyclonic spray chamber

Microwave instrument	**MARS-5 from CEM instrument, UK**
Power	800 W
%	100
Ramp	3.0 minutes
Temperature control	55°C for 20 min and 60°C for 20 min
Pressure (psi)	0.00
Temperature programme	65°C for 40 minutes
Stage	2

Table 1.
Microwave operating conditions for hair samples digestion.

were connected to the heated steel transfer capillary line connected ICP torch, and this enabled continuous aspiration of a standard thallium solution (25μgl^{-1}). This configuration allowed optimization of instrument performance and simultaneous measurement of ^{203}Tl and ^{205}Tl for mass bias correction during the chromatographic run [9]. Operating conditions for the GC-ICP-MS coupling system are listed in **Table 2**.

2.2 Reagents and standards

All chemicals used were of analytical reagent grade unless stated otherwise. Tetramethylammonium hydroxide (TMAH, 25% w/w in water) and ethylmercury chloride were purchased from Alfa Aesar (UK). Methanol, sodium acetate, and acetic acid glacial (super grade) were purchased from VWR (BDH, UK). Sodium tera-n-propylborate ($NaBpR_4$, ≥98% purity) was purchased from Chemos GmbH (Germany). 2, 2, 4 trimethylpentane (isooctane, spectrophotometric grade, ≥99% purity) and methylmercury (II) chloride were purchased from Sigma Aldrich (UK). Inorganic mercury (In-Hg) standard solution for ICP (934 ± 3.0 mg/kg) was purchased from Fluka (UK).

The derivatization solution was prepared by dissolving 1 g of sodium tetrapropylborate ($NaBpR_4$) in 100 ml of deionized water. The solution was stored at −20°C in a refrigerator and protected from light. Buffer acetate (0.1 M) in deionized water was prepared by mixing 0.1 M sodium acetate solution (90 ml) with 0.1 M acetic acid

ICP-MS Instrument	**Agilent 7500 series**
Hg isotope acquired	199, 200, 201, 202
Acquired mode	Time-resolved
Dwell time	0.035 sec/point
RF power	1380 W
RF matching	1.53 V
Sample depth	6.3 mm
Torch-H	1.1 mm
Torch-V	0.4 mm
Carrier gas	Argon/0.79 l min^{-1}
Makeup gas	Argon/0.17 l min^{-1}
Extract 1	−2 V
Internal standard	Tl (25 ppb)
Nebulizer pump flow rate	0.20 rps
Spray chamber temperature	2 °C
GC Instrument	Agilent HP 6850
Injection	Split/splitless—1 μl
Oven program	50 °C (1 min), 50 °C/min 220°C (5 min
Carrier gas	Helium
Transfer line temp	200°C
GC injector temp	220°C

Table 2.
GC and ICP-MS operating parameters.

solution (410 ml) and adjusted the final volume to 1000 ml (1 L) with deionized water and adjusting to pH 3.9. Inorganic mercury working standard solutions (1.0 and 10 mg/kg as Hg) were prepared from appropriate dilution of inorganic mercury standard stock solution (934 ± 3.0 mg/kg). Enriched inorganic mercury (1.0 mg/kg ^{199}In-Hg199 as Hg) and enriched methylmercury (1.0 mg/kg ^{201}MeHg as Hg) working solutions were prepared from appropriate dilution of their standard stock solutions. Milli-Q quality water (Millipore) was used throughout.

2.3 Derivatization by sodium tetrapropylborate and analytical procedures

Blank (TMAH) and hair samples with a low level (0.98 mg/kg) of In-Hg and one hair sample from ICL with a high level of In-Hg (1000 mg/kg) were spiked with the same amount of ^{201}MeHg and ^{199}InHg (double spike, 70 μl from 1.0 ppm of each enriched Hg standard). The spiked and un-spiked hair samples were digested using a microwave device. The spiked sample solution (blank and digested spiked hairs) and un-spiked hair solution samples in cleaned and dried glass vials (1 ml of each) were then adjusted to pH 3.9 with acetate buffer, and then, 1 ml of 1% $NaBPr_4$ was added in the glass vials for derivatization to form the corresponding peralkylated mercury (Hg) species such as.

$$Hg^{2+} + 2NaB\,(C_3H_5)_4 \rightarrow Hg\,(C_3H_5)_2 + 2Na^{+} + 2B\,(C_3H_5)_3 \quad (3)$$

$$CH_3Hg^{+} + NaB\,(C_3H_5)_4 \rightarrow CH_2HgC_3H_5 + Na^{+} + B\,(C_3H_5)_3 \quad (4)$$

Extraction of derivatized Hg species (peralkylated Hg) was done by vigorous shaking for 5 min with 1 ml isooctane, the isooctane extract was afterwards centrifuged for 10 min at 3000 rpm, and then, the extracted Hg species into isooctane layer were transferred to GC vials and analyzed with a coupling of GC-ICP-MS. In addition, the conc. Normal abundance In-Hg, conc. Enriched In-Hg^{199}, and hair sample from chlor alkali plant with similar Hg conc. to both In-Hg conc. (about 1000 mg/kg) and different concentrations of normal abundance In-Hg standard solutions (20, 40, 60, 80, and 100 mg/kg) were derivatized and extracted as described above. The samples were diluted after derivatization and extraction steps (D.F 1:10 for the concentrated Hg standards and chlor alkali hair sample).

3. Results and discussion

3.1 Hg isotope ratio calculation for spiked and un-spiked samples

The mercury isotope ratios (IR) were calculated for four measured Hg isotopes (199, 200, 201, 202) in spiked, un-spiked blank (TMAH), hair samples, and Hg standard solution (normal abundance In-Hg and enriched ^{199}In-Hg) to compare the formation of mercury artifact in spiked and un-spiked samples during propylation with $NaBPr_4$. The artifact formation of monomethy lmercury (Me-Hg) and ethylmercury (EtHg) from inorganic mercury (In-Hg) was observed during propylation with $NaBPr_4$ for spiked blank with enriched ^{199}InHg and enriched ^{199}In-Hg standard solution comparing with un-spiked blank (**Figures 1** and **2**). The extent of artifact formation for the organomercury compounds was in the order: Et-Hg > -Me-Hg > Hg(0). Moreover, the artifact formation of monomethyl–ethylmercury

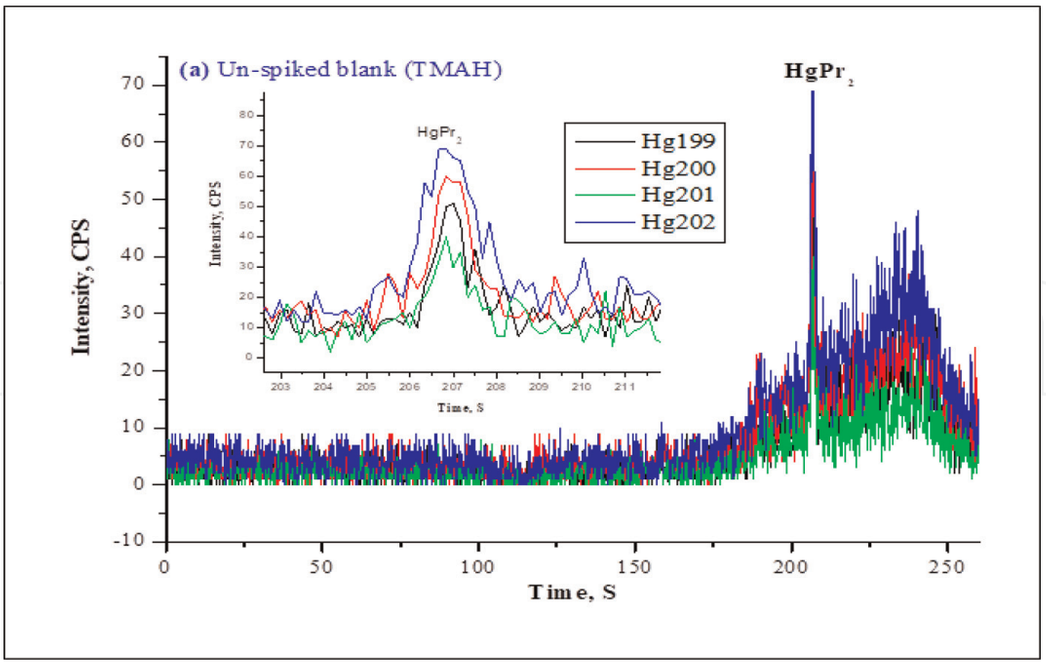

Figure 1.
Typical chromatogram of un-spiked blank (1.0 ml TMAH) obtained during derivatization using $NaBPr_4$.

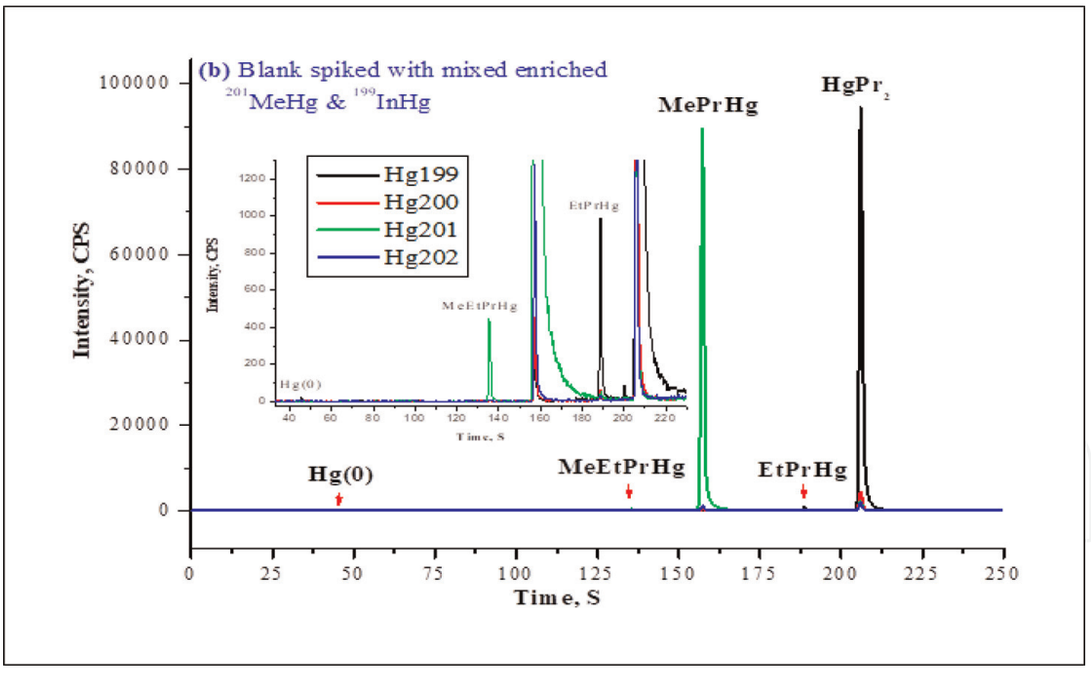

Figure 2.
Typical chromatogram of spiked blank (TMAH) with mixed enriched ^{201}MeHg & ^{199}In-Hg standard solutions obtained during derivatization using $NaBPr_4$.

(MeEt-Hg) from methylmercury was observed also during propylation with $NaBPr_4$ for **a** spiked blank (TMAH) with mixed enriched mercury standards (^{201}Me-Hg and ^{199}In-Hg) as shown in **Figure 2**.

For mercury isotope ratio (IR) calculation results for un-spiked blank (TMAH) compared with spiked blank with the same amount of mixed enriched mercury standards (^{201}MeHg and ^{199}InHg, 70 µl from 1.0 ppm of each into 1 ml of TMAH) after

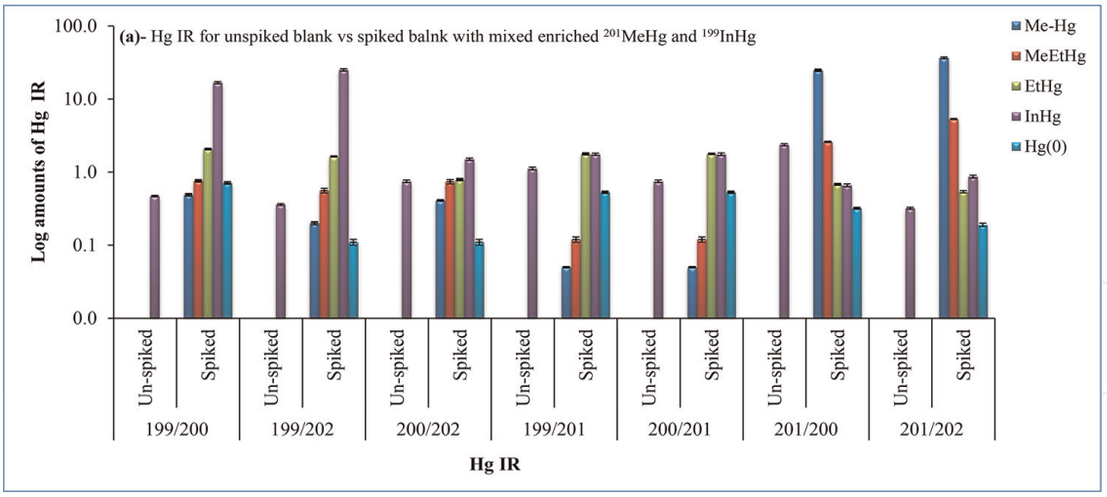

Figure 3.
Compression of calculated mercury isotope ratios (Hg-IR) in un- spiked blank with spiked blank with mixed enriched ^{201}MeHg & ^{199}In-Hg standard solutions during the derivatization using $NaBPr_4$.

propylation with $NaBPr_4$ as shown in **Figure 3**. It can be seen that the signal in each of the mercury isotopic ratios (Hg199/200, Hg199/201, Hg199/202, Hg200/201, Hg200/202, and Hg201/200) for spiked blank is increased in the order: In-Hg > Et-Hg > -MeEt-Hg > Me-Hg, but for un-spiked blank was observed only the similar ratio for all Hg isotope ratios for In-Hg only. This means that the artifact formation of an organomercury compound is increased with increasing amounts of inorganic mercury (InHg) when spiked the blank with enriched mercury standards (^{201}Me-Hg and ^{199}In-Hg) and propylated with $NaBPr_4$. However, this is indicating that the artifact formation of MeHg and EtHg from a high concentration of inorganic mercury is caused by $NaBPr_4$.

In addition, it can be seen from **Figure 4** that the results of IR calculation for spiked blank (TMAH) with enriched ^{199}InHg are similar to those calculated in

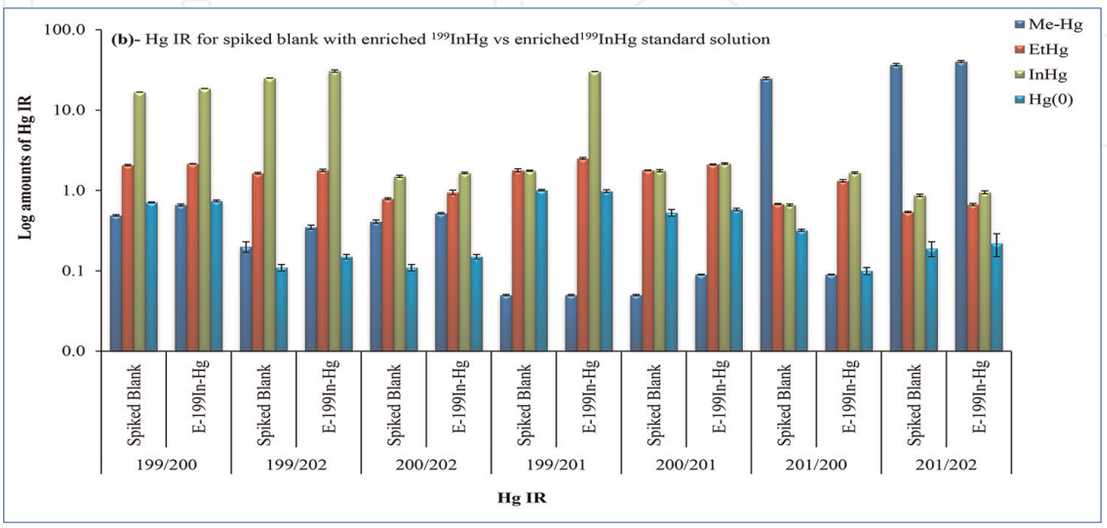

Figure 4.
Compression of calculated mercury isotope ratios (Hg-IR) in spiked blank with enriched ^{199}InHg spiked blank versus enriched ^{199}In-Hg standard solution during derivatization using $NaBPr_4$.

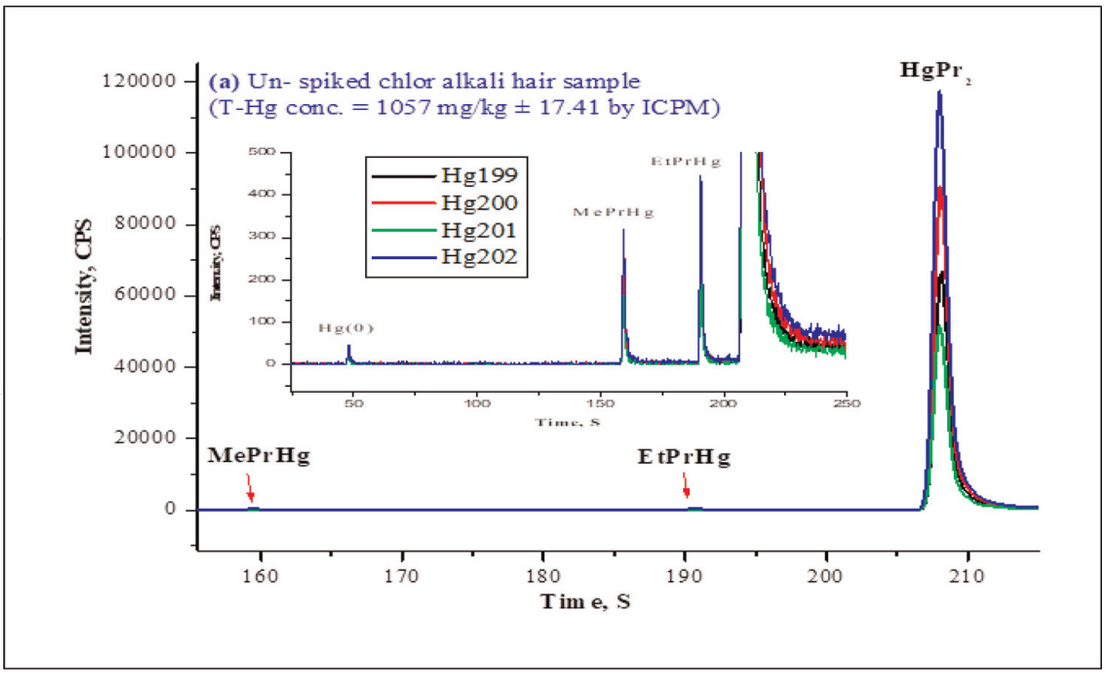

Figure 5.
Typical chromatogram of un- spiked chlor alkali hair sample (CA hair sample with T-Hg conc. = 1000 mg/kg) obtained during derivatization using $NaBPr_4$.

enriched ^{199}InHg standard solution after propylation by $NaBPr_4$. Moreover, it can be deduced from IR calculations that MeHg and EtHg artifact creation originate exclusively from InHg, but MeEtHg artefact formation is not detected. This indicates that MeEtHg artefacts only originate from enriched ^{201}MeHg when the blank or sample is spiked with high levels of enriched ^{201}MeHg (greater than 50 l of 1.0 ppm to 1.0 ml of blank or 0.01.

For un-spiked hair samples from one chlor alkali plant with a high concentration of inorganic mercury (In-Hg) and normal abundance inorganic mercury (In-Hg) with similar Hg concentration to those found in selected chlor alkali plants hair sample, the mercury isotope ratios (IR) were calculated for four measured Hg isotopes (199, 200, 201, and 202) same as in spiked, un-spiked blank (TMAH), and enriched ^{199}InHg. The observation of artifact formation of methylmercury (MeHg) and ethylmercury (EtHg) from inorganic mercury (InHg) was similar to those found in the spiked blank with enriched ^{199}InHg and enriched ^{199}InHg standard solution during propylation with $NaBPr_4$ (**Figures 5** and **6**). However, as shown in **Figures 5** and **6**, the transformation of MeHg and EtHg from In-Hg in both chlor alkali hair and normal abundance InHg is similar and the extent of artifact formation for the organomercury compounds was in the order: EtHg>MeHg>Hg(0).

Mercury isotope ratio (IR) calculation results for un-spiked chlor alkali hair compared with spiked same hair sample with mixed enriched mercury standards (^{201}MeHg and ^{199}InHg, 70 ul from 1.0 ppm of each into 1 ml of TMAH) and normal abundance InHg after propylation with $NaBPr_4$ as shown in **Figures 7** and **8** below showed similar amounts of all mercury isotope ratio (IR) in both spiked and un-spiked hair samples. This indicates that the methylmercury (MeHg) and ethylmercury (EtHg) compounds are artifacts of high amounts of inorganic mercury in hair samples owing to the propylation with NaBPr4 and are not reliant on the spiking quantity of

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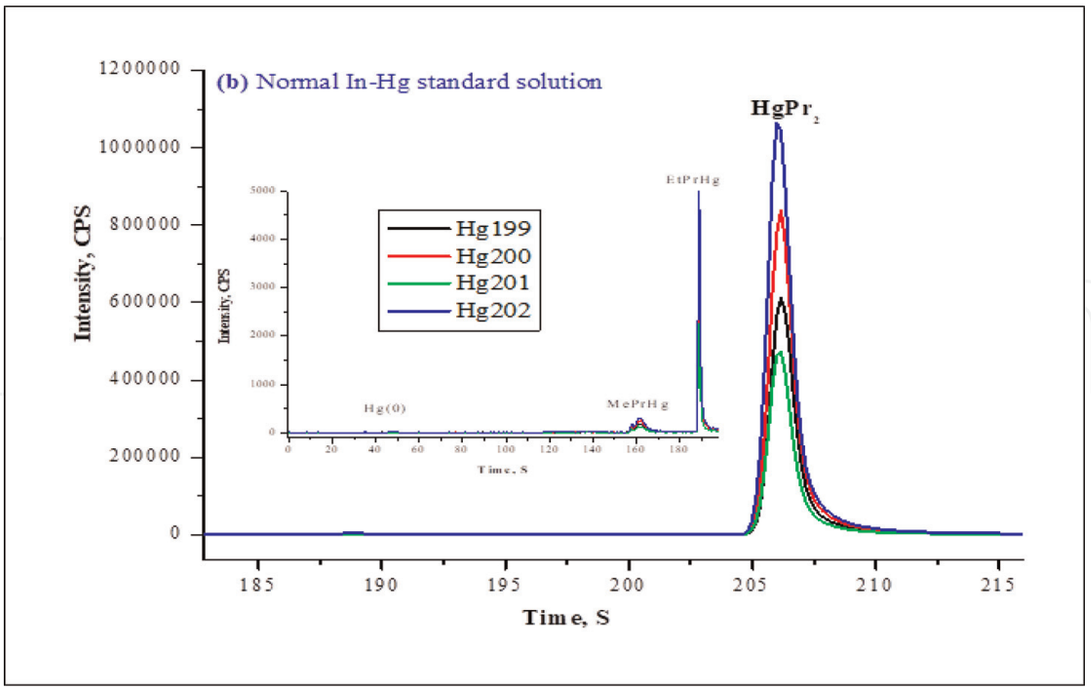

Figure 6.
Typical chromatogram of normal abundance In-Hg (1000 mg/kg as Hg^{2+}) obtained during derivatization using $NaBPr_4$.

enhanced mercury standards, as well as depending on the purity of the propylation reagent.

Moreover, when Hg isotope ratios (IR) calculating results in spiked normal hair the sample (control hair sample) is compared to spiked chlor alkali hair (both hair samples were spiked with the same quantity of mixed enriched ^{201}MeHg and ^{199}InHg standard solutions), as shown in **Figure 9** below, it can be noted that the Hg isotope ratio calculation findings are identical in both spiked hair samples except for In-Hg isotope ratios (IR).

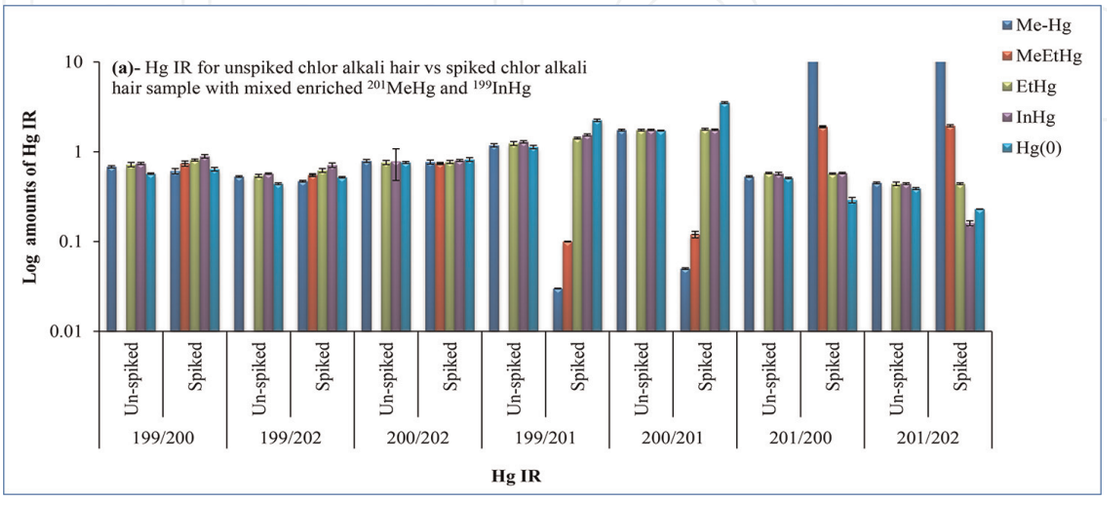

Figure 7.
Compression of calculated mercury isotope ratios (Hg-IR) in un- spiked CA hair spiked CA hair mixed enriched ^{201}MeHg & ^{199}In-Hg standards during derivatization using $NaBPr_4$.

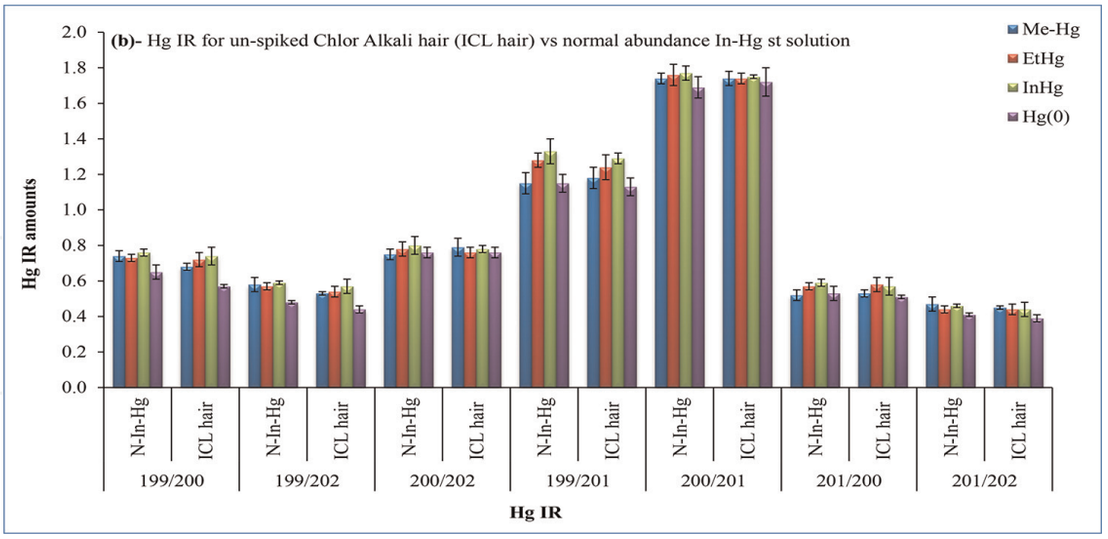

Figure 8.
Compression of calculated mercury isotope ratios (Hg-IR) in un- spiked CA hair verses similar In-Hg concentration of normal abundance In-Hg standard solution during derivatization using $NaBPr_4$.

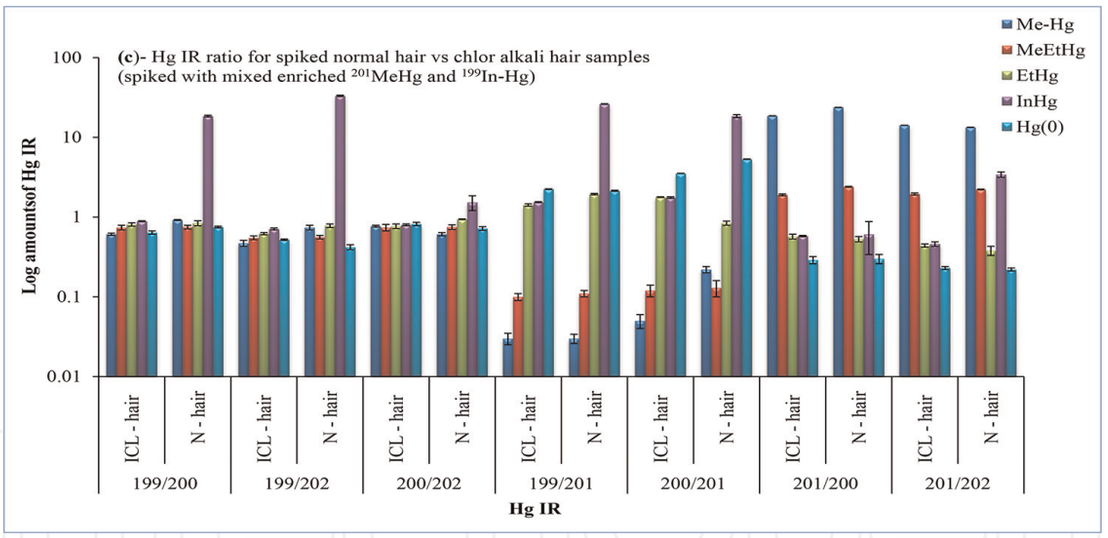

Figure 9.
Comparing the compression of (Hg-IR) in spiked normal hair (N-hair) with mixed enriched 199In-Hg and 201MeHg standards to spiked CA-hair mixed standard solutions utilizing NaBPr4.

3.2 Methyl (MeHg) and Ethylmercury (EtHg) percentage (%) artifact formation in the blank (TMAH) and the hair samples during derivatization by $NaBPr_4$

To calculate the percentage amounts of artifacts formation of methyl and ethylmercury in spiked blank and hair samples, the normal abundance inorganic mercury standard solution, and enriched inorganic mercury (^{199}In-Hg) during derivatization by NaBPr4, the artifact percentage (%) calculation was done as follows:

3.2.1 Comparison of MeHg and EtHg % artifact formation in un-spiked blank (TMAH) with spiked blank and enriched abundance ^{199}InHg

It can be observed from **Table 3** and **Figure 10** that the average artifact formation of MeHg was 0.012% for both spiked blank (TMAH) with the low amount of enriched ^{199}InHg (70 ul of 1 mg/kg of standard) and high amount of ^{199}InHg standard solution (20 mg/kg), while there is no artifact present in un-spiked blank (TMAH). Also, the average artifact formation of EtHg (0.82%) was higher about 68 times than the average artifact formation of MeHg in both spiked blank and enriched InHg standard solution.

3.2.2 Comparison of MeHg and EtHg % artifact formation in un-spiked chlor alkali hair sample with spiked CA hair and normal abundance InHg standard solution

To compare the rate of MeHg and EtHg percentage (%) artifact formation in chlor alkali hair sample (un-spiked and spiked CA hair sample) and normal

Sample ID	MeHg and EtHg % artifact formation				
	Hg (0) (%)	MeEtHg (%)	MeHg (%)	EtHg (%)	InHg (%)
Un-spiked blank (TMAH)	0.00	0.00	0.00	0.00	100
Spiked blank with enriched ^{199}InHg	0.00	0.00	0.012	0.82	99.10
Enriched ^{199}InHg standard solution	0.00	0.00	0.012	0.82	99.10

Table 3.
Comparison of MeHg and EtHg % artifact formation in the un-spiked blank (TMAH) with spiked blank and enriched abundance InHg standard during derivatization using $NaBPr_4$.

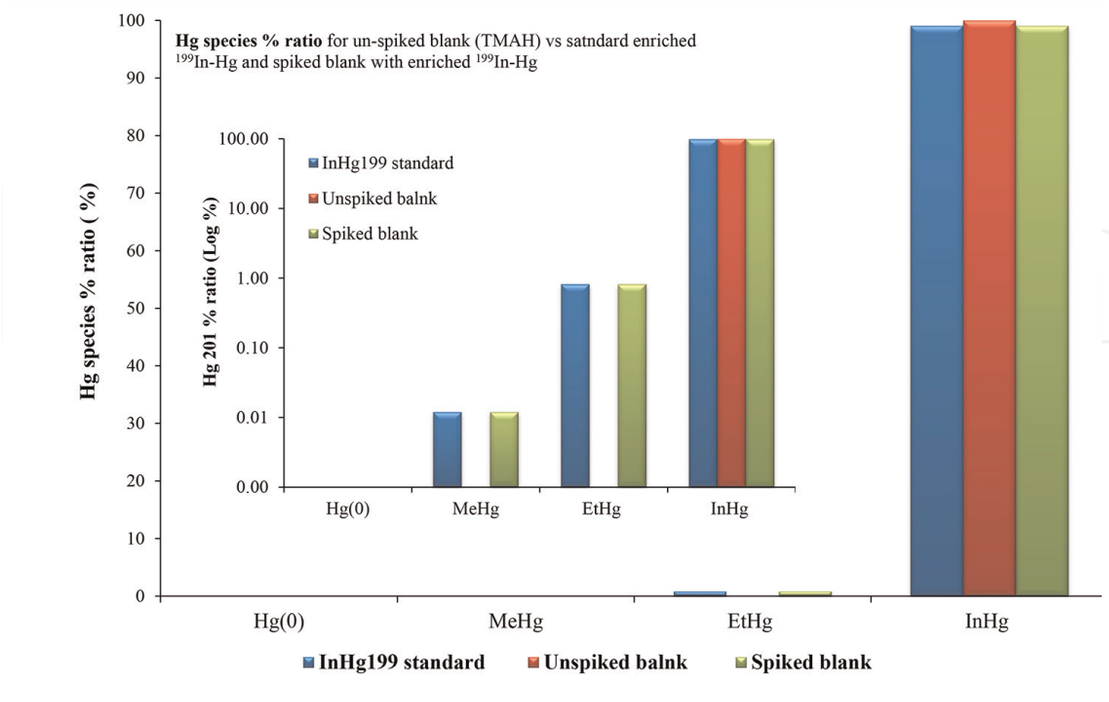

Figure 10.
Compression of calculated mercury isotope ratios (Hg-IR) in spiked blank with enriched ^{199}InHg the spiked blank versus enriched ^{199}In-Hg standard solution during derivatization using $NaBPr_4$.

Sample ID	MeHg and EtHg % artifact formation				
	Hg (0) (%)	MeEtHg (%)	MeHg (%)	EtHg (%)	InHg (%)
Un-spiked CA hair	0.012	0.000	0.220	0.76	99.04
Spiked CA hair with mixed enriched ^{201}MeHg &^{199}InHg	0.013	0.24	0.232	0.76	98.78
Normal abundance InHg standard solution	0.010	0.000	0.012	0.76	99.20

Table 4.
Comparison of MeHg and EtHg % artifact formation in un-spiked CA hair with spiked CA hair and normal abundance InHg during derivatization using $NaBPr_4$.

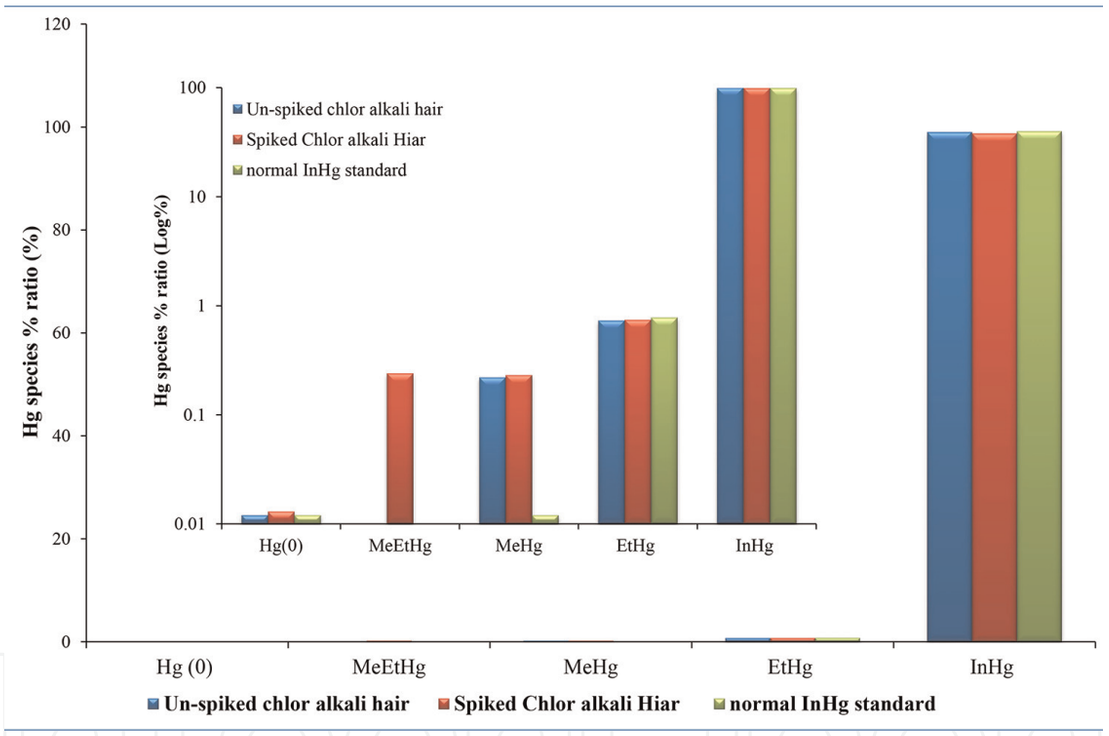

Figure 11.
Comparison of MeHg and EtHg % artifact formation in un-spiked CA hair versus spiked CA hair with mixed enriched ^{201}MeHg & ^{199} InHg standards and the same amount of normal abundance InHg during derivatization using $NaBPr_4$.

abundance In-Hg standard solution, further calculations were done as shown in **Table 4** and **Figure 11**, and the percentage results were similar to those found in spiked and spiked blank. However, it can be observed that the rate of MeHg % artifact formation is increased by the factor of 0.012% (from 0.220% in un-spiked CA hair to 0.232% in spiked CA hair sample with mixed enriched ^{201}MeHg and ^{199}InHg), while the percentage artifact formation rates of EtHg were constant (0.76% for both of each). In addition, for normal abundance standard solution, the percentage (%) artifact formation of MeHg and EtHg was recorded similar to those found in spiked chlor

alkali hair sample (CA hair). Moreover, the % arifact formation rate of MeEtHg (0.24%) was recored only in spiked CA hair sample with mixed enriched ^{201}MeHg and ^{199}InHg standard solutions (70 ul of 1.0 ppm from each standard). This is meaning that the artifact formation of MeEtHg comes from enriched ^{201}MeHg in comparison with un-spiked CA hair and normal abundance In-Hg standard solution.

3.3 Influence artifact formation on determining of methylmercury (Me-Hg) in biological (hair) and environmental samples

To estimate the influence of the artifact on the methyl mercury (MeHg) and ethylmercury (Et-Hg) analysis, the artifact during extraction and derivation was investigated by comparing of un-spiked chlor alkali hair sample (CA hair, with a high level of In-Hg) with different concentration of normal abundance In-Hg (20, 40, 60, 100 mg/kg In-Hg as Hg^{2+}). The un-spiked chlor alkali hair sample (0.02 g) was digested in 5 ml TMAH using microwave device under temperature programme of 55° C for 20 min and 60°C for 20 min. The extract for CA hair and five normal abundance In-Hg standard solutions were then derivatized with $NaBPr_4$, extracted, and analyzed with the same procedure as described in Section 2.3. From the results as shown in **Table 5** and **Figure 12**, it can be seen that there is no substantial enhancement of artifact of Me-Hg and Et-Hg observed as compared to the amounts of artifact MeHg and EtHg shown in **Tables 3** and **4** and **Figures 10** and **11**. This result indicates that the percentage of artifact formation of methylmercury (MeHg) and ethylmercury (EtHg) from all different concentrations of normal abundance In-Hg standard solution was constant at the rates of 0.012 and 0.80%, respectively, during derivatization using $NaBPr_4$. However, as shown in **Table 5** and **Figure 12**, the most of MeHg found in the un-spiked CA hair extract more likely originated from the CA hair sample. Taking 0.012% as average constant formation rate for artifact MeHg, the CA hair sample showed that artifact MeHg might result in less than 6% of the measured MeHg value. Moreover, **Table 5** and **Figure 12** indicate that all EtHg found in un-spiked CA and five different concentrations of normal abundance In-Hg standard solutions are artifacts at the same artifacts formation percentage (0.80%) from the high level of In-Hg in the samples during derivatization using $NaBPr_4$. Despite of this, this method is useful to measure the actual amount of MeHg in hair samples by subtracting of percentage MeHg artifact formation constant ratio (0.012%) from that found in **c**hlor alkali hair samples or any samples contains high levels of inorganic mercury (In-Hg).

Sample ID	**% Artifact formation from InHg**			
	Hg (0) (%)	**MeHg (%)**	**EtHg (%)**	**InHg (%)**
In-Hg (20 mg/l)	0.012	0.012	0.80	99.18
In-Hg (40 mg/l)	0.011	0.012	0.81	99.12
In-Hg (60 mg/l)	0.012	0.012	0.80	99.14
In-Hg (80 mg/l)	0.011	0.012	0.80	99.13
In-Hg (100 mg/l)	0.012	0.013	0.81	99.12
Un-spiked chlor alkali hair	0.011	0.220	0.77	99.09

Table 5.
Comparison of MeHg and EtHg % artifact formation in un-spiked CA hair with different amounts of normal abundance InHg during derivatization using $NaBPr_4$.

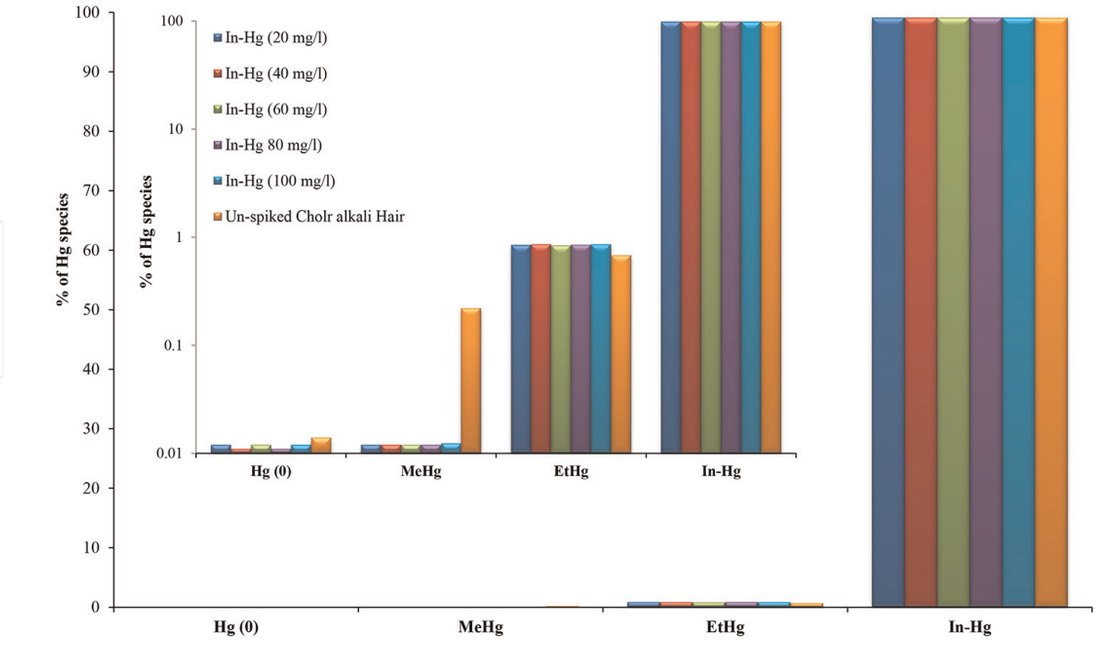

Figure 12.
Comparison of MeHg and EtHg % artifact formation in un-spiked CA hair versus different concentrations of normal abundance InHg during derivatization using $NaBPr_4$.

4. Conclusions

Artificial mercury speciation and quantification errors of organomercury compounds are caused by the artifact generation of organomercury compounds like Me-Hg and Et-Hg during the analytical methods. There were obvious artifact formation of methylmercury (Me-Hg) and ethylmercury (Et-Hg) compounds from a high level of inorganic mercury (more than 20 mg/kg) during NaBPr4 derivatization, and so this highly depends on the amount of inorganic mercury (Hg2+) present in the derivatization solution and the purity of sodium tetra (n-propyl) borate (NaBP). The high rate of artifact Et-Hg formation (0.76 to 0.81% of high-level Hg2+ present) seriously impairs Et-Hg analysis. This demonstrates that the sodium tetra (n-propyl) borate (NaBPr4) reagent is not suitable for the analysis of EtHg when inorganic mercury (In-Hg) concentrations in samples are higher than 20 mg/kg. The rate of methylmercury (MeHg) artifact creation is low and steady (0.012% of InHg present), and it has no impact on the analysis of methylmercury (MeHg) since the MeHg artifact ratio can be removed from the observed value of MeHg in the samples. However, the EtHg peak must be visible in the samples' chromatograms to do the mathematical correction for MeHg measurement. Additionally, the majority of the inorganic mercury (In-Hg) from the solid samples can be removed using acid leashing procedures before the derivatization step to prevent the formation of organomercury compounds (Me-Hg and Et-Hg) as an artifact during the derivatization process using NaBPr4 [29, 30].

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References

[1] Li P, Feng X, Qiu G, Shang L, Li G. Human hair mercury levels in the Wanshan mercury mining area, Guizhou Province, China. Environmental Geochemistry and Health. 2009;**31**(6): 683-691

[2] Zhang Z, Wang Q, Zheng D, Zheng N, Lu X. Mercury distribution and bioaccumulation up the soil-plant-grasshopper-spider food chain in Huludao City, China. Journal of Environmental Sciences. 2010;**22**(8): 1179-1183

[3] Rahman GMM, Fahrenholz T, Kingston HM. Application of speciated isotope dilution mass spectrometry to evaluate methods for efficiencies, recoveries, and quantification of mercury species transformations in human hair. Journal of Analytical Atomic Spectrometry. 2009;**24**(1):83-92

[4] Delgado A, Prieto A, Zuloaga O, de Diego A, Madariaga JM. Production of artifact methylmercury during the analysis of certified reference sediments: Use of ionic exchange in the sample treatment step to minimise the problem. Analytica Chimica Acta. 2007;**582**(1):109-115

[5] Kim SA, Jeon CK, Paek DM. Hair mercury concentrations of children and mothers in Korea: Implication for exposure and evaluation. Sci Total Environ. 2008;**402**(1):36-42

[6] Agusa T, Kunito T, Iwata H, Monirith I, Tana TS, Subramanian A, et al. Mercury contamination in human hair and fish from Cambodia: Levels, specific accumulation and risk assessment. Environmental Pollution. 2005;**134**(1):79-86

[7] Chen S, Chou S, Hwang D. Determination of methylmercury in fish using focused microwave digestion following by Cu2+ addition, sodium tetrapropylborate derivatization, n-heptane extraction, and gas chromatography–mass spectrometry. Journal of Chromatography A. 2004; **1024**(1–2):209-215

[8] Bancon-Montigny C, Yang L, Sturgeon RE, Colombini V, Mester Z. High-yield synthesis of milligram amounts of isotopically enriched methylmercury (CH3198HgCl). Applied Organometallic Chemistry. 2004;**18**(2):57-64

[9] Krupp EM, Donard OFX. Isotope ratios on transient signals with GC-MC-ICP-MS. International Journal of Mass Spectrometry. 2005;**242**(2-3): 233-242

[10] Mizanur Rahman GM, Skip Kingston HM, Bhandari S. Synthesis and characterization of isotopically enriched methylmercury (CH3201Hg+). Applied Organometallic Chemistry. 2003;**17**(12): 913-920

[11] Martín-Doimeadios RCR, Krupp E, Amouroux D, Donard OFX. Application of isotopically labeled methylmercury for isotope dilution analysis of biological samples using gas chromatography/ ICPMS. Analytical Chemistry. 2002; **74**(11):2505-2512

[12] Tseng CM, Amouroux D, Brindle ID, Donard OFX. Field cryofocussing hydride generation applied to the simultaneous multi-elemental determination of alkyl-metal(loid) species in natural waters using ICP-MS detection. Journal of Environmental Monitoring. 2000;**2**(6):603-612

[13] Gao Y, Shi Z, Long Z, Wu P, Zheng C, Hou X. Determination and speciation of mercury in environmental and biological samples by analytical

atomic spectrometry. Microchemical Journal. 2012;**103**(0):1-14

[14] Reyes LH, Mar JLG, Hernández-Ramírez A, Peralta-Hernández JM, Barbosa JMA, Kingston HMS. Microwave assisted extraction for mercury speciation analysis. Microchimica Acta. 2011;**172**(1):3-14

[15] Rodrigues JL, Alvarez CR, Fariñas NR, Berzas Nevado JJ, Barbosa F Jr, Rodriguez Martin-Doimeadios RC. Mercury speciation in whole blood by gas chromatography coupled to ICP-MS with a fast microwave-assisted sample preparation procedure. Journal of Analytical Atomic Spectrometry. 2011; **26**(2):436-442

[16] Clough R, Truscatt J, Belt ST, Hywel Evans E, Fairman B, Catterick T. Isotope dilution ICP-MS for speciation studies. Applied Spectroscopy Reviews. 2003; **38**(1):101-132

[17] Hill SJ, Pitts LJ, Fisher AS. High-performance liquid chromatography–isotope dilution inductively coupled plasma mass spectrometry for speciation studies: An overview. TrAC Trends in Analytical Chemistry. 2000;**19**(2–3): 120-126

[18] Rodríguez-González P, Marchante-Gayón JM, García Alonso JI, Sanz-Medel A. Isotope dilution analysis for elemental speciation: A tutorial review. Spectrochimica Acta Part B: Atomic Spectroscopy. 2005;**60**(2):151-207

[19] Qvarnström J, Frech W. Mercury species transformations during sample pre-treatment of biological tissues studied by HPLC-ICP-MS. Journal of Analytical Atomic Spectrometry. 2002; **17**(11):1486-1491

[20] Lambertsson L, Lundberg E, Nilsson M, Frech W. Applications of enriched stable isotope tracers in combination with isotope dilution GC-ICP-MS to study mercury species transformation in sea sediments during in situ ethylation and determination. Journal of Analytical Atomic Spectrometry. 2001;**16**(11):1296-1301

[21] Hintelmann H, Evans RD. Application of stable isotopes in environmental tracer studies—Measurement of monomethylmercury (CH3Hg+) by isotope dilution ICP-MS and detection of species transformation. Fresenius' Journal of Analytical Chemistry. 1997;**358**(3):378-385

[22] Yang L, Colombini V, Maxwell P, Mester Z, Sturgeon RE. Application of isotope dilution to the determination of methylmercury in fish tissue by solid-phase microextraction gas chromatography–mass spectrometry. Journal of Chromatography A. 2003; **1011**(1–2):135-142

[23] Horvat M, Bloom NS, Liang L. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples: Part 1 Sediments. Analytica Chimica Acta. 1993;**281**(1):135-152

[24] Horvat M, Liang L, Bloom NS. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples: Part II water. Analytica Chimica Acta. 1993;**282**(1):153-168

[25] Cai Y, Jaffé R, Jones R. Ethylmercury in the soils and sediments of the Florida everglades. Environmental Science & Technology. 1997;**31**(1):302-305

[26] Huang J. Artifact formation of methyl- and ethyl-mercury compounds from inorganic mercury during

derivatization using sodium tetra(n-propyl)borate. Analytica Chimica Acta. 2005;**532**(2):113-120

[27] Mao Y, Liu G, Meichel G, Cai Y, Jiang G. Simultaneous speciation of monomethylmercury and monoethylmercury by aqueous phenylation and purge-and-trap preconcentration followed by atomic spectrometry detection. Analytical Chemistry. 2008;**80**(18):7163-7168

[28] Demuth N, Heumann KG. Validation of methylmercury determinations in aquatic systems by alkyl derivatization methods for GC analysis using ICP-IDMS. Analytical Chemistry. 2001;**73**(16):4020-4027

[29] Bermejo-Barrera P, Verdura-Constenla EM, Moreda-Piñeiro A, Bermejo-Barrera A. Rapid acid leaching and slurry sampling procedures for the determination of methyl-mercury and total mercury in human hair by electrothermal atomic absorption spectrometry. Analytica Chimica Acta. 1999;**398**(2–3):263-272

[30] Gao Y, De Galan S, De Brauwere A, Baeyens W, Leermakers M. Mercury speciation in hair by headspace injection–gas chromatography–atomic fluorescence spectrometry (methylmercury) and combustion-atomic absorption spectrometry (total Hg). Talanta. 2010;**82**(5):1919-1923