

Comparison of GC-ICP-MS and HPLC-ICP-MS for Speciation of Mercury in Blood

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Introduction

The potential impact of Hg in the environment and its effects on human health is a topical subject in today's society. Mercury, ubiquitous in the environment due to natural and anthropogenic sources, has been linked to central nervous system (CNS) diseases due to its toxic nature. Organomercurials, in particular methylmercury (MeHg⁺) are known to be exceptionally toxic due to their increased mobility and ability to accumulate in fatty tissues. This phenomenon has been repeatedly reported in marine fish where bioaccumulation and bioamplification can lead to mg Hg/kg MeHg⁺ concentrations in certain fish species. MeHg⁺ can be efficiently transported in the blood stream and accumulates readily in body lipids and cerebella neurons of the CNS. Additionally, MeHg⁺ crosses the blood-brain and blood-placenta membranes posing a direct risk to unborn babies.



Efforts to curb any possible CNS damage due to MeHg⁺ from fish consumption are ongoing in the form of maximum dose recommendations from a number of health and food safety agencies. These recommendations are aimed primarily at pregnant women, nursing mothers and young children, where a link between MeHg⁺ in the bloodstream and damage to the developing nervous system has previously been established.

Research currently focuses on the accumulation of MeHg⁺ from food sources, its mobility in the human body and its role in the cause of disease and brain damage. To investigate and the potential risk of MeHg⁺ in a more direct manner, a method for its determination in blood would be a valuable tool for the toxicological and medical community. However, determination is problematic due to the low concentrations of MeHg⁺ and the complex nature of the blood matrix which can interfere with both the sample preparation procedure and the analysis. In this presentation, two approaches to mercury speciation, GC and HPLC-ICP-MS were developed and scrutinized for lyophilized blood samples (which contain either heparin or EDTA as anticoagulant). Evaluation of the methods was performed using the NIST bovine blood SRM 966 and/or recovery experiments

GC-ICP-MS

A Thermo Scientific TRACE GC Ultra™ was coupled to a Thermo Scientific XSERIES 2 quadrupole ICP-MS using the commercially available coupling kit with unique dual mode sample introduction system (Figure 1). Chromatographic and instrumental parameters are shown in Table 1.

FIGURE 1. TRACE GC Ultra coupled to the XSERIES 2 ICP-MS.



TABLE 1. GC and ICP-MS parameters.

Column	Thermo Scientific TRACE™ TR-5 GC Column, 30 m x 0.25 mm ID, 25 µm
Injection mode	PTV, splitless
Injection port temperature	250 °C with ramp to 400 °C
Injection volume	1 µL
Carrier gas flow	He @ 3 mL min ⁻¹
GC oven parameters	50°C (1 min), ramp at 30 °C/min to 300 °C (1 min)
Nebulizer and spray chamber	Concentric with impact bead (free aspiration)
Nebulizer gas	0.35 L min ⁻¹
Additional gas	Ar @ 600 mL min ⁻¹
Forward power	1400 W

Sample Preparation

An ethylmercury (EtHg⁺) internal standard equivalent to a final concentration of 10 ppb was added to 1 mL of NIST 966 level 2 or 250 mg of lyophilized blood. Standard addition or recovery spikes of Hg species were added to the samples at this point; therefore prior to extraction. 5 mL TMAH was added to each blood sample and extracted for 1 hr in an ultrasonic bath. 3 extraction blanks were prepared in the same manner. After extraction, 2 mL of extract was taken for derivatisation. The extract aliquot was added to 5 mL of 0.1 M ammonium acetate buffer at pH 5 and the pH was adjusted to pH 5 using acetic acid. 1 mL hexane and 1 mL 4% NaBPr₄ were added to the vial and agitated for 5 mins. The vials were centrifuged to encourage separation of the aqueous and solvent phase. The hexane phase was passed through a florisil SPE cartridge and approximately 100 µL was recovered and transferred to an insert in a GC vial.

Results

The NIST 966 level 2 blood extract with internal standard spike is compared to a 1 µg/L standard in Figure 2. From comparison of the internal standard recovery in the NIST 966 blood and the recovery in a blank or standard we can approximate the derivatisation efficiency to be around 16% when the blood matrix is present.

FIGURE 2. Chromatograms of (a.) 1 µg/L Hg species (b.) NIST 966 level 2.

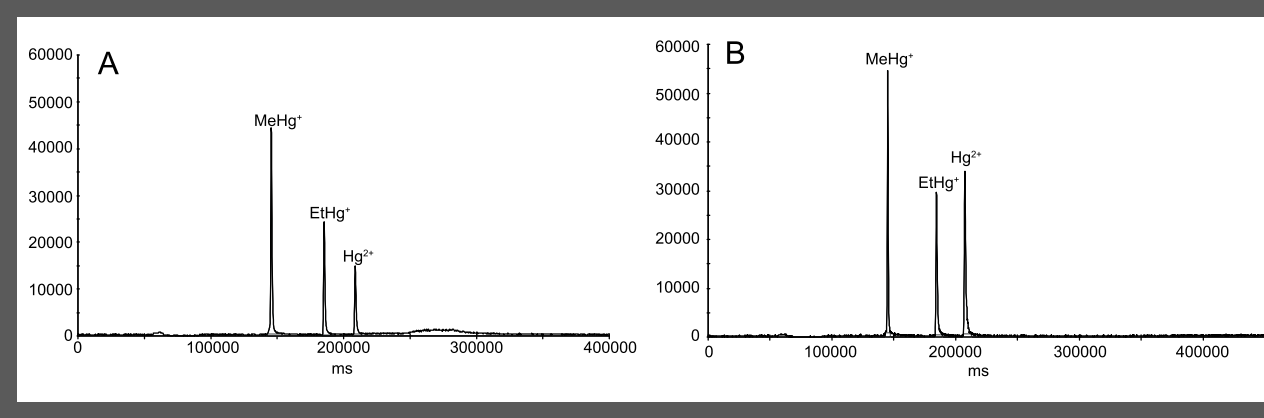


FIGURE 3. Standard Addition Calibration for NIST 966 level 2.

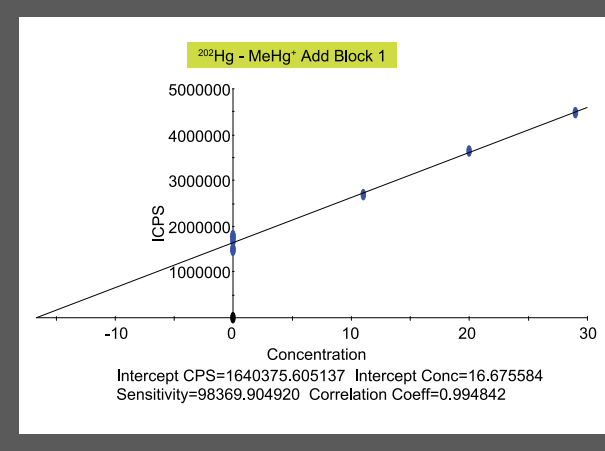
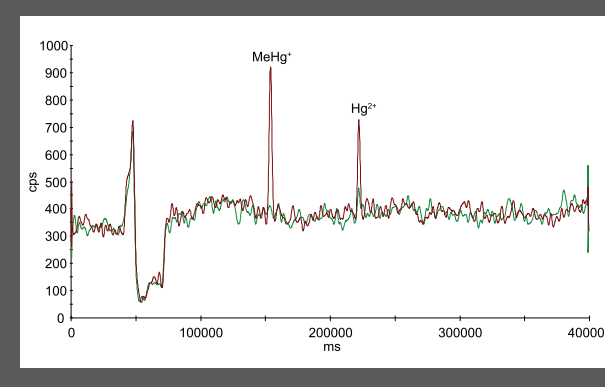


FIGURE 4. Lyophilized blood extract (green) with overlay of lyophilized blood extract with a 1 µg/L Hg species spike (brown).



Low recoveries due to the complex matrix may be exacerbated due to the lyophilized nature of blood or the anticoagulant present. An alternative analytical approach which omits the derivatization step such as HPLC-ICP-MS may be an alternative.

HPLC-ICP-MS

A Thermo Scientific Accela™ HPLC pump was coupled to an XSERIES 2 ICP-MS (Figure 5). The chromatographic and instrumental parameters are shown in Table 2.

FIGURE 5. Accela HPLC coupled to the XSERIES 2 ICP-MS.

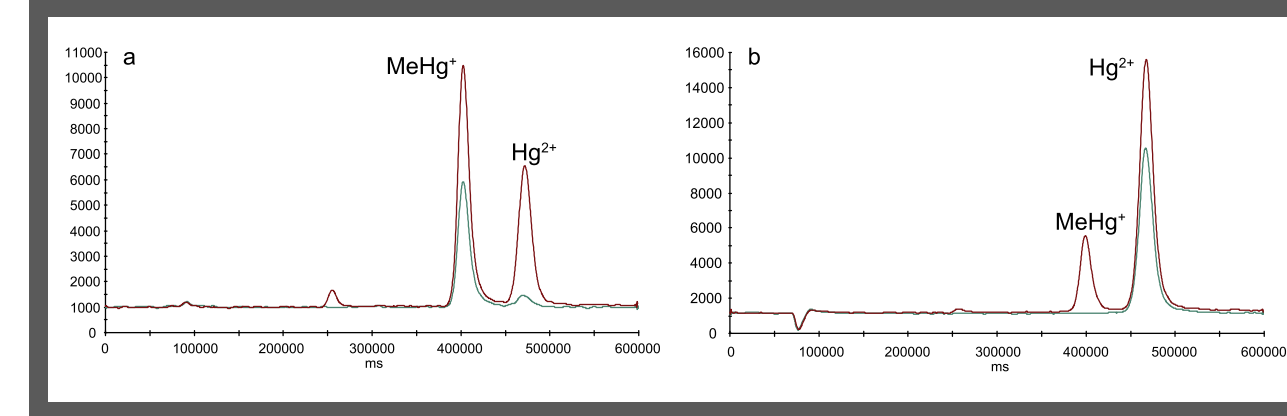


TABLE 2. HPLC and ICP-MS parameters.

Column	Thermo Scientific Hypersil GOLD™, 150 x 4.6 mm, 5 µm
Injection volume	100 µL
Flow rate	1 mL min ⁻¹
Isocratic elution	60 mM ammonium acetate, 0.1% mercaptoethanol, 1% MeOH
Forward power	1400 W
Nebulizer, spray chamber	Glass concentric, Peltier cooled impact bead

The described HPLC-ICP-MS methodology for Hg speciation has been previously developed and applied to fish extracts and urine (Figure 6). DORM-2 was used to validate the method for MeHg⁺ with a 96% recovery of the certified value of 4.47 µg Hg/g.

FIGURE 6. Mercury speciation in (a) DORM-2 and (b) urine (overlays show spikes of Hg species).



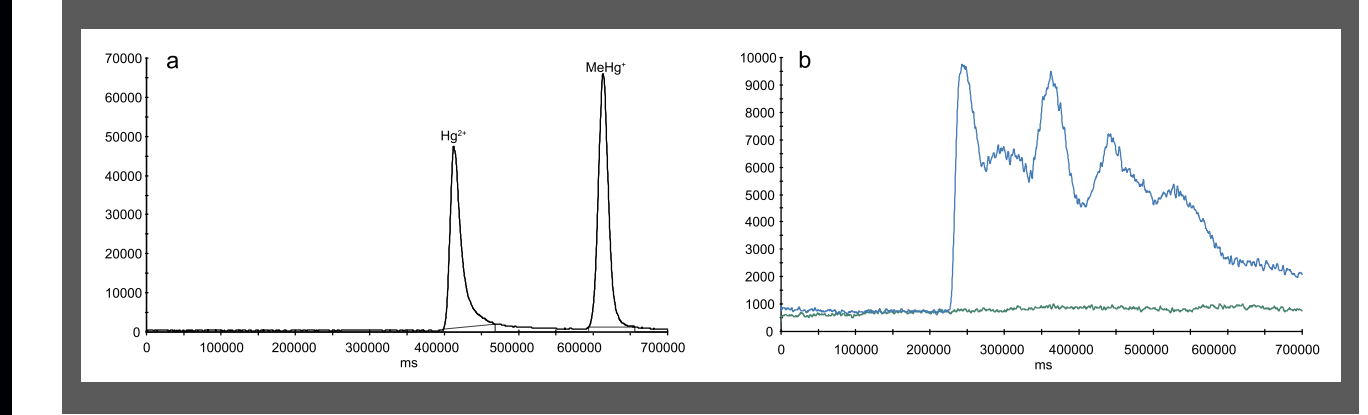
Sample Preparation

Please refer to sample preparation in GC section. The sample preparation for HPLC does not require the derivatisation step. For the initial evaluation, only the lyophilized blood samples were analysed and recovery spikes were performed after the extraction step.

Results

The lyophilized blood extract (containing heparin) is compared in Figure 7 to a set of Hg-containing standards. Any Hg species in the blood extract are not eluted on the HPLC column. However, total Hg concentrations in the lyophilized blood sample extracts determined using the XSERIES 2 give concentrations around 4 µg/L. A post extraction spike of MeHg⁺ and Hg²⁺ was therefore performed, but the chromatographic elution of these species did not follow the normal elution profile. It is suspected that blood matrix constituents and possibly the anticoagulants which are strong ligands are forming complexes with the Hg species, disrupting their elution.

FIGURE 7. Chromatographic elution of (a) Hg-containing standards at 5 µg/L and (b) lyophilized blood extract (green) with overlay of lyophilized blood extract spiked with 5 µg/L Hg-species (blue).



Conclusions

The methods developed so far for the speciation of Hg in blood samples are not yet applicable to lyophilized blood samples. Although the chromatographic methods work well for standards and a number of other matrices, the complex blood matrix is problematic for derivatization and elution. For successful blood analysis, continuing development of the sample preparation is needed.

The GC method was validated for the NIST bovine blood 966, although the derivatization efficiency was only 16% due to the complex matrix. The LOD for MeHg⁺ in NIST 966 is estimated as 0.4 µg/L based on the LOD obtained from 3 replicate blank injections and taking into account the low derivatization efficiency for blood.

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