Sample Preparation and Analytical Methodology for Routine Mercury Speciation Analysis in Environmental Samples

Solving Matrix Dependent Problems in Mercury Speciation Analysis

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Sample Matrix and Typical Mercury Species Concentrations

<table>
<thead>
<tr>
<th>Water Sample Matrix</th>
<th>$\text{Hg}^{2+}$ (ng/L)</th>
<th>$\text{MeHg}^+$ (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>0.5-12</td>
<td>0.02 -1.5</td>
</tr>
<tr>
<td>Sea Water</td>
<td>0.1-5</td>
<td>0.01-0.5</td>
</tr>
<tr>
<td>Estuarine/Polluted Water</td>
<td>10-130</td>
<td>0.5-100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solid Sample Matrix</th>
<th>$\text{Hg}^{2+}$ (ng/g)</th>
<th>$\text{MeHg}^+$ (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>0.5-50</td>
<td>5-1000</td>
</tr>
<tr>
<td>Sediments</td>
<td>5-1000</td>
<td>0.05-5</td>
</tr>
</tbody>
</table>
1. GC-ID-ICP-MS
   a. Sediments
   b. Seawater
   c. Biological Tissues

2. HPLC-ICP-MS
**GC-ICP-MS**

With Dual Mode Introduction System

- Three legged GC-ICP-MS torch
- Autotune & Performance; reporting with aqueous solution
- Gas or solution analyses without reconfiguring the interface
- On-line addition of aqueous internal standards (Thallium - NIST 977)
- Robust wet plasma conditions for GC-ICP-MS analysis
Speciation of Mercury by GC-ICP-MS
Derivatization of Mercury Species

It is necessary to derivatise the Mercury species to render them volatile for separation by GC.

1. Hg species in ammonium acetate buffer (0.1 M), pH 3.9
2. add 1 mL 1% NaBEt₄
3. add 1 mL isoctane
4. shake for 5 min to complete derivatisation
5. transfer aliquot of isoctane into GC vial
6. Inject 1 uL
Stability of GC-ICP-MS analyses for repeat injections of Hg species over 7h

**Mercury Speciation**
**GC-ICP-MS Figures of Merit**

Run time: 6 min
Sample throughput: 180/day
BEC: 0.001 ng/g
LOD: 0.015 ng/g
GC-Species Specific Isotope Dilution-ICP-MS

Natural Abundances

Isotopically Enriched

Sample + Isotopically Enriched Standard

Modified Ratio

\[ R^{202\text{Hg}/199\text{Hg}} \]

\[ c'w'A_r (R'Y' - X') \]

\[ wA_r'(X - RY) \]

Conditions Required for Species Specific Isotope Dilution

- Isotopically Enriched Species:
  - \( \text{Me}^{202\text{Hg}}^+, 199\text{Hg}^2\).

- Optimisation of GC-ICP-MS:
  - Separation/Detection Parameters

- Optimisation of sample prep:
  - Extraction/Derivatisation (pH, reagents)
GC-Species Specific Isotope Dilution-ICP-MS

‘Double Spike’

Retention time/s

Intensity/cps

\[ R = 200 \]

\[ R = 199 \]

Natural Hg

\[ \text{MeHg}^+ \]

\[ + \text{Me}^{202}\text{Hg}^+ \]

\[ \text{Hg}^{2+} \]

\[ + {^{199}\text{Hg}}^+ \]

199 200 202

199 200 202

199 200 202

199 200 202

199 200 202

199 200 202
1. GC-ID-ICP-MS
   a. Sediments
   b. Seawater
   c. Biological Tissues
2. HPLC-ICP-MS
Determination of \( \text{MeHg}^+ \) in Sediments by GC-ID-ICP-MS

- Sediments tend to have a high concentration of \( \text{Hg}^{2+} \) in relation to \( \text{MeHg}^+ \)
- \( \text{MeHg}^+ \) represents only about 0.5 - 2 % total Hg

<table>
<thead>
<tr>
<th>CRM</th>
<th>( \text{MeHg}^+ ) (ng/g)</th>
<th>( \text{Hg}^{2+} ) (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR 580 (Estuarine)</td>
<td>75.5</td>
<td>132 000</td>
</tr>
<tr>
<td>IAEA 356 (Polluted Marine)</td>
<td>5.87</td>
<td>7620</td>
</tr>
<tr>
<td>IAEA 405 (Estuarine)</td>
<td>5.9</td>
<td>810</td>
</tr>
</tbody>
</table>

- !!! Artificial formation of \( \text{MeHg}^+ \) during sample prep procedure
- Even if only a very small percentage (0.02 - 0.03 %) of \( \text{Hg}^{2+} \) is methylated during sample prep, this can cause an overestimation of 30 - 80 % of \( \text{MeHg}^+ \) in sediment
Determination of MeHg+ by SIDMS in Sediments
Sample preparation

**Spike-extraction procedure**

- **sediment** + **spike** $^{201}$MMHg
- +2 ml methanol
- Agitation 12H
- Dry under $N_2$ 3H
- +10 ml HNO$_3$ 6N
- Microwave 2min 40W

**Direct derivatization**

- Ethylation
- Ethylation in diluted conditions

**Derivatization**

- Solvent extraction $CH_2Cl_2$
- Ethylation
- Grignard
Determination of MeHg by SIDMS in Sediments
Application to CRMs

**CRMs**

**CRM 580**

- Ethylation: 75.5 ± 3.7 ng MeHg+/g
- Ethylation in diluted conditions: 132 ± 3 µg Hg²+/g

**IAEA 356**

- Ethylation: 5.87 ± 0.89 ng MeHg+/g
- Ethylation in diluted conditions: 7.62 ± 0.65 µg Hg²+/g

**IAEA 405**

- Ethylation: 5.90 ± 0.57 ng MeHg+/g
- Ethylation in diluted conditions: 0.81 ± 0.04 µg Hg²+/g

**Direct derivatization**

- Ethylation
- Ethylation in diluted conditions

**Derivatization after CH₂Cl₂ extraction**

- Ethylation
- Grignard
**Prevent Artefact Formation:**

Extract with \( \text{CH}_2\text{Cl}_2 + \text{HCl} \), 10 mins

Addition of HCl:
- \( \text{Hg}^{2+} \rightarrow (\text{HgCl}_4)^{2-} \) IONIC
- \( \text{MeHg}^+ \rightarrow \text{MeHgCl} \) NEUTRAL

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**Sample Prep Protocol**

- Weigh 1 g sediment
- Add aliquot of isotopically enriched species
  - \( \text{MeHg}^+ \) enriched with \( ^{202}\text{Hg} \) and \( \text{Hg}^{2+} \) enriched with \( ^{199}\text{Hg} \)
- Add 5 mL nitric acid (6 N)
- Microwave extraction for 3 min at 45 W
- 2 mL extract + 1.5 mL \( \text{CH}_2\text{Cl}_2 \) + 100 \( \mu \)L HCl, shake for 10 min
- Transfer 1 mL aliquot of solvent to 5 mL ammonium acetate buffer (0.1 M), pH 3.9
- Adjust pH to 3.9 with ammonium hydroxide (25 %)
- Add 1 mL 1% NaBEt4 and 1 mL isooctane
- Shake for 5 min to complete derivatisation
- Transfer aliquot of isooctane into GC vial
Effect of $\text{CH}_2\text{Cl}_2$ Extraction on Speciation of Mercury in Sediments

IAEA 405 with solvent extraction

IAEA 405 without solvent extraction
Determination of MeHg⁺ in Sediments by GC-ID-ICP-MS

BCR 580 with solvent extraction

\[
c = \frac{c'w'A Rangers (RY'- X')}{wA Rangers (X - RY)}
\]

<table>
<thead>
<tr>
<th>ng/g</th>
<th>Blank</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0275</td>
<td>77.13</td>
</tr>
<tr>
<td>2</td>
<td>0.0156</td>
<td>76.29</td>
</tr>
<tr>
<td>3</td>
<td>0.0185</td>
<td>76.41</td>
</tr>
<tr>
<td>Mean</td>
<td>0.02</td>
<td><strong>76.61</strong></td>
</tr>
<tr>
<td>St. Dev.</td>
<td>0.008</td>
<td><strong>0.45</strong></td>
</tr>
<tr>
<td>RSD %</td>
<td>41.5</td>
<td>0.6</td>
</tr>
<tr>
<td>LOD</td>
<td>0.023</td>
<td></td>
</tr>
</tbody>
</table>
1. GC-ID-ICP-MS
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2. HPLC-ICP-MS
Simultaneous Mercury and Tin Speciation
Optimization of the Sample Prep

Derivatization
pH

Salt Effect
Synthetic sea water spiked with 1 ng/L

MeHg$^+$  Hg$^{2+}$  TBT

Recovery (%)

<table>
<thead>
<tr>
<th>Salinity</th>
<th>13‰</th>
<th>35‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeHg$^+$</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Hg$^{2+}$</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>TBT</td>
<td>140</td>
<td>140</td>
</tr>
</tbody>
</table>

External calibration
Species specific isotope dilution
Simultaneous Mercury and Tin Speciation
Optimization of the Sample Prep - Derivatization Agent

Ethylation in MQ water
Propylation in MQ water
Ethylation in salt water
Propylation in salt water

Normalized sensitivity

Hg compounds higher sensitivity with propylation
BuSn compounds same sensitivity
Determining MeHg⁺ by SIDMS in Seawater
Sample Prep Protocol
Preconcentration of Hg Species

- Weigh 100 mL water (river, estuarine, sea)
- Add aliquot of isotopically enriched species
  - (MeHg⁺ enriched with ²⁰²Hg and Hg²⁺ enriched with ¹⁹⁹Hg)
- Add 5 mL ammonium acetate buffer (0.1 M), pH 3.9 (or pH 4.9 for simultaneous speciation)
- Adjust pH with ammonium hydroxide (25 %)
- Add 1 mL 1% NaBPr₄ and 100 µL - 1 mL isooctane
- Shake for 5 min to complete derivatisation
- Transfer aliquot of isooctane into GC vial

**HANDY TIP:**

- Once derivatisation is complete, add milli-Q water.
  - The isooctane solvent layer in the neck of the bottle is easier to access with a pipette.

Hg species in 100 mL water → 100 µL isooctane
Simultaneous Mercury and Tin Speciation
Natural Sample of Seawater

Ethylation

MeHg$^+$ < DL

Propylation

MeHg$^+$ = 0.042ng/L
1. GC-ID-ICP-MS
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2. HPLC-ICP-MS
Mercury Speciation
Determination of MeHg\(^+\) in Fish
Sample Prep Protocol

BCR 464 (tuna fish) or fresh fish

• Freeze dry fresh fish (preservation, ease of handling)
• Grind freeze dried fish tissue with pestle and mortar
• Weigh 250 mg BCR 464 or freeze dried fish
• add aliquot of isotopically enriched species
  • (MeHg\(^+\) enriched with \(^{202}\text{Hg}\) and Hg\(^2+\) enriched with \(^{199}\text{Hg}\))
• add 5 mL TMAH (25 %)
• microwave extraction for 2 min at 40 W
• transfer 1 mL aliquot of extract to 5 mL ammonium acetate buffer (0.1 M), pH 3.9
• adjust pH to 3.9 with acetic acid
• add 1 mL 1% NaBEt\(_4\) and 1 mL isoctane
• shake for 5 min to complete derivatisation
• centrifuge and transfer aliquot of isoctane into GC vial
Mercury Speciation
Determination of MeHg⁺ in Fish

Marine Fish

Freshwater Fish

Freshwater Perch
Analytical Approach

150 Fish or Fish Based Meals

**Total Hg**
- Fish - freeze-dried and homogenized
- Extraction - $\text{HNO}_3 + \text{H}_2\text{O}_2$, sonication or heating, dilution
- ICP-MS

**MeHg$^+$ and Hg$^{2+}$**
- Extraction
- Fish - TMAH, microwave
- Derivatisation - adjust pH, propylating agent, iso-octane
- GC-ID-ICP-MS

**MASS BALANCE** for samples where MeHg$^+$ $\sim$ [Hg]
Relation Between MeHg$^+$ and Total Hg in Commercial Fish Samples and DORM-2

Precision: % of MeHg$^+$ values that fall within 100 ± 15 % [Hg]

<table>
<thead>
<tr>
<th>Sample</th>
<th>% of results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>54</td>
</tr>
<tr>
<td>DORM-2</td>
<td>100</td>
</tr>
</tbody>
</table>

DORM-2 method validation: $[\text{MeHg}^+] = 4.24 \pm 0.24 \, \mu g/g$ (95% of certified value)

Precision is Sample Dependent
- Homogeneity
- Sample matrix
- Sample prep
Sources of Errors in Speciation/Sample Prep

- Problem 1. Matrix such as fats, proteins, other elemental species (Se, S)
- Problem 2. Extraction preferential towards native or spiked MeHg⁺
- Problem 3. Transformation preferential to native Hg species

Other sources of error:

- Batch analysis: many samples to be analysed in certain time limit
- For ID, the spike added is based on a ‘typical’ species concentration
1. GC-ID-ICP-MS
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2. HPLC-ICP-MS
Speciation of Mercury
Reversed Phase HPLC-ICP-MS

HPLC parameters
- HPLC column: Hypersil GOLD (150 x 4.6 mm, 5µm)
- Isocratic elution:
  - 60 mM ammonium acetate,
  - 5% methanol,
  - 0.01% 2-mercaptoethanol
- Flow rate: 1.5 mL/min
- Inj. vol: 100 µL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run time</td>
<td>10 min</td>
</tr>
<tr>
<td>Sample throughput</td>
<td>120/day</td>
</tr>
<tr>
<td>BEC</td>
<td>0.007 ng/g</td>
</tr>
<tr>
<td>LOD</td>
<td>0.05 ng/g</td>
</tr>
</tbody>
</table>

Retention time/min

Intensity/cps
Speciation of Mercury in Biological Tissues
Validation of Technique with CRM

DORM-2 (plus 2 ppb spike)

Fish sample prep
Extraction: + 5 mL TMAH Microwave 3 min 40W Analytical methodology (4 min.)

Method validation: [MeHg⁺] = 4.27 ± 0.12 μg/g (96% of certified value)
Speciation of Mercury in Liquid Samples

No Sample Preparation Needed

**Estuarine Water (plus 1 ppb spike)**

Mobile Phase flow rate
= 1.0 mL/min

**Urine (plus 1 ppb spike)**

Mobile Phase flow rate
= 1.5 mL/min
Conclusions and Perspectives

- **GC-ID-ICP-MS:**
  - GC-ICP-MS instrumentation is automated, reproducible, sensitive
  - Instrumentation now commercially available

- **Sample prep:**
  - Needs to be carefully optimised for each matrix as precision of results is often dependent on sample matrix

- **Isotope Dilution:**
  - Eliminates need for external caln. or standard addition
  - Quantitative recovery is not necessary
  - Rearrangement reactions detected and corrected

- **HPLC-ICP-MS an alternative technique for Hg speciation:**
  - HPLC - adequate LOD and sample throughput
  - Minimal sample prep, even for samples with complex matrix

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Does Routine Hg Speciation Exist Yet?

Method development and validation with CRMs
Alternative analytical approaches
THANKS !!!!

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