

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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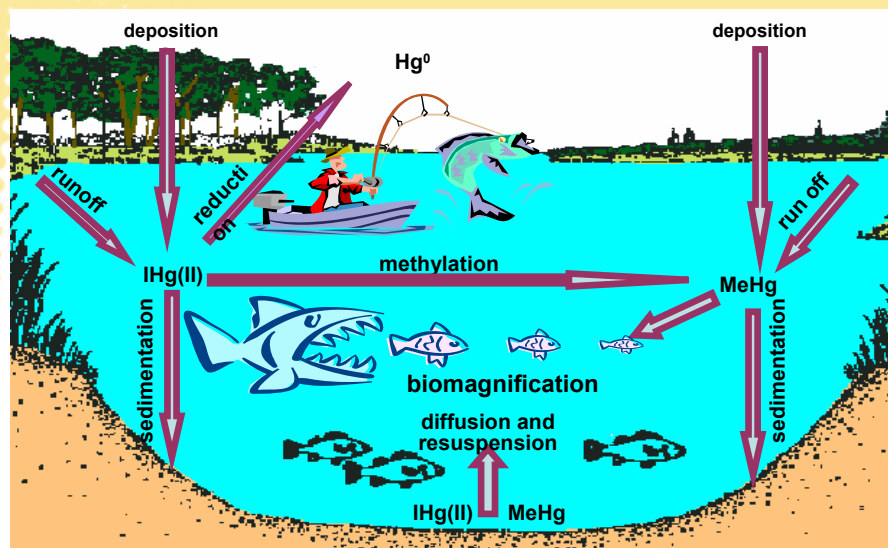
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Sample Matrix and Typical Mercury Species Concentrations

| Water Sample Matrix | Hg ²⁺ (ng/L) | MeHg ⁺ (ng/L) |
|--------------------------|-------------------------|--------------------------|
| Fresh Water | 0.5-12 | 0.02 -1.5 |
| Sea Water | 0.1-5 | 0.01-0.5 |
| Estuarine/Polluted Water | 10-130 | 0.5-100 |
| Solid Sample Matrix | Hg ²⁺ (ng/g) | MeHg ⁺ (ng/g) |
| Fish | 0.5-50 | 5-1000 |
| Sediments | 5-1000 | 0.05-5 |



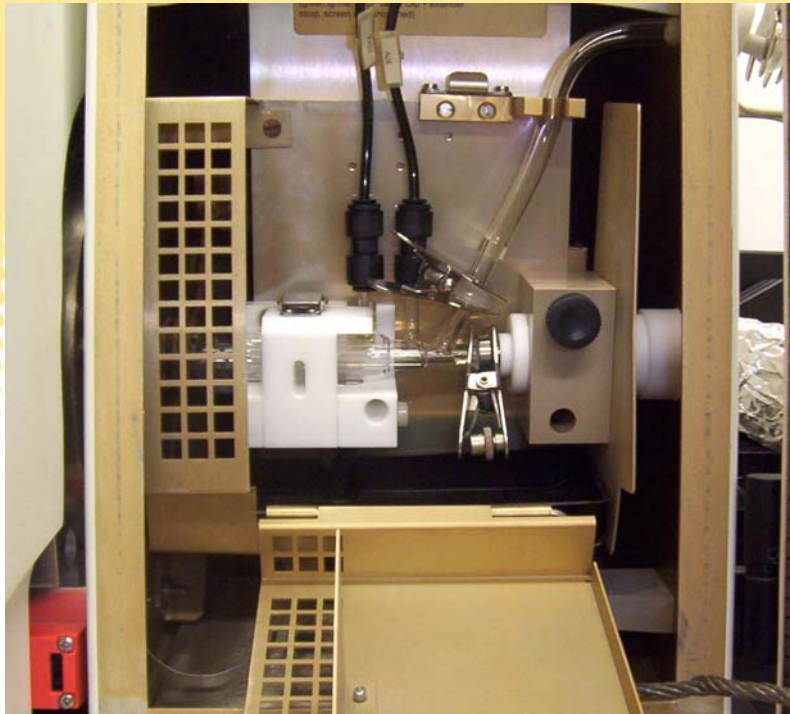


1. GC-ID-ICP-MS
 - a. Sediments
 - b. Seawater
 - c. Biological Tissues
2. HPLC-ICP-MS

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

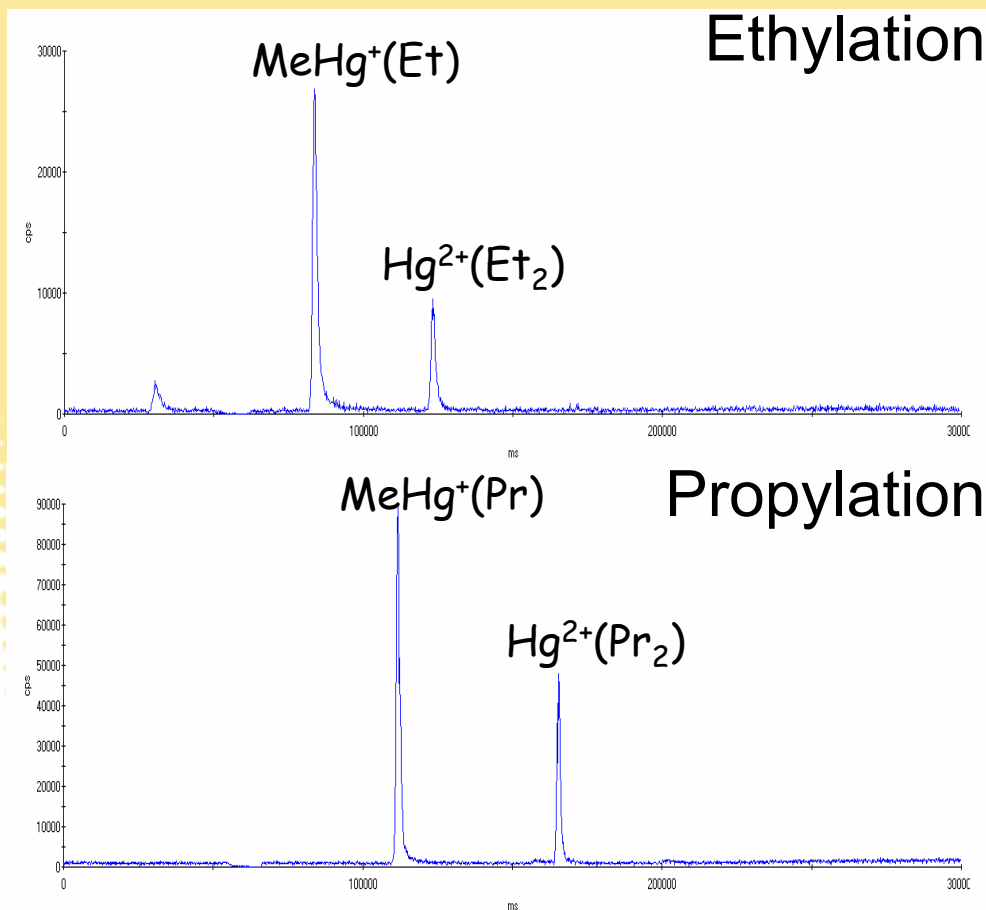


Speciation of Mercury by GC-ICP-MS

Derivatization of Mercury Species

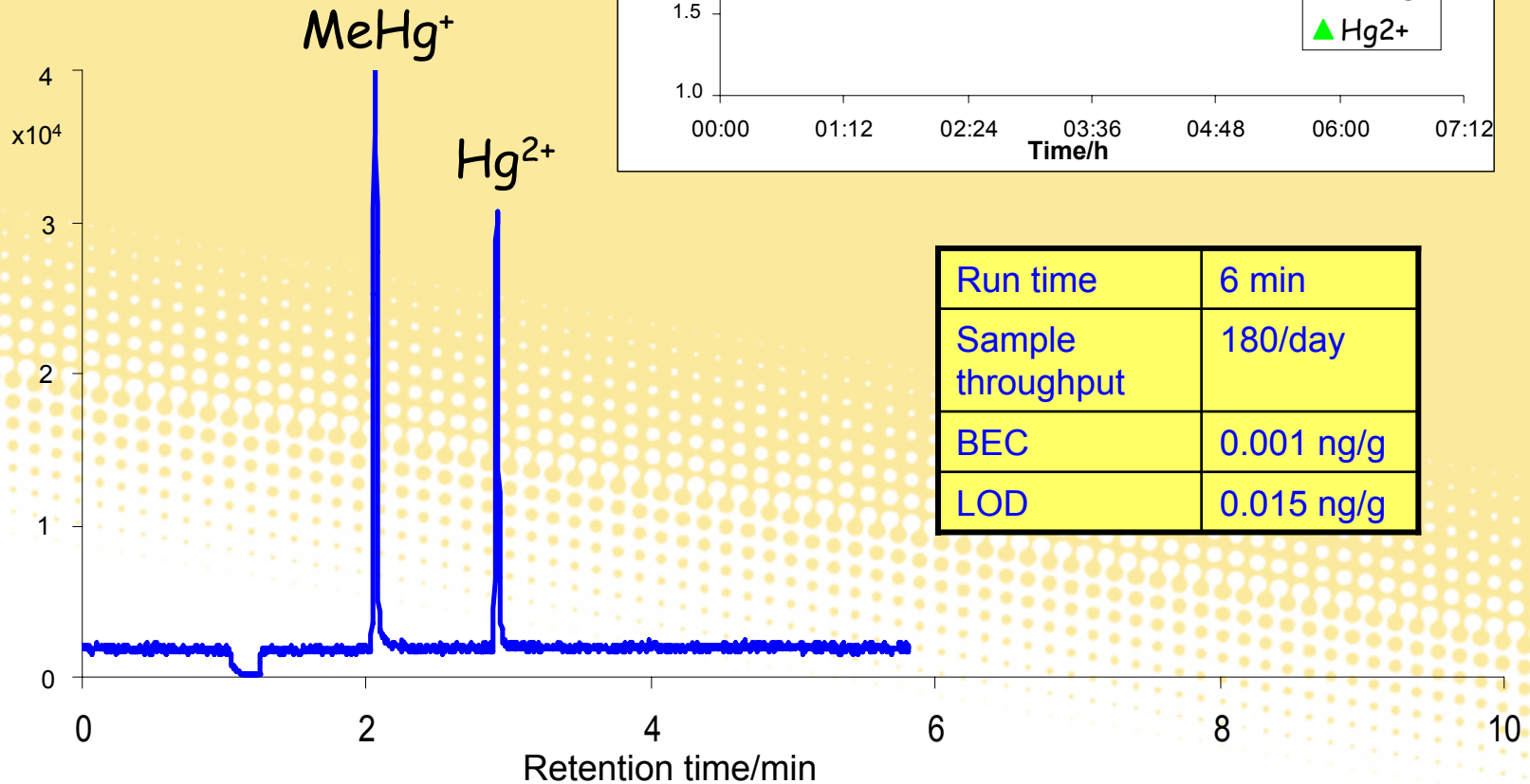
It is necessary to derivatise the Mercury species to render them volatile for separation by GC

1. Hg species in ammonium acetate buffer (0.1 M), pH 3.9
2. add 1 mL 1% NaBEt₄
3. add 1 mL isooctane
4. shake for 5 min to complete derivatisation
5. transfer aliquot of isooctane into GC vial
6. Inject 1 μ L



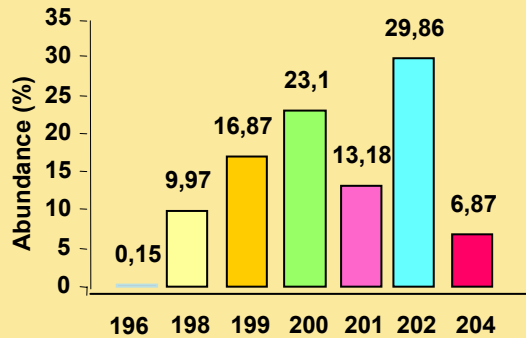
Mercury Speciation GC-ICP-MS Figures of Merit

Stability of GC-ICP-MS
analyses for repeat injections
of Hg species over 7h

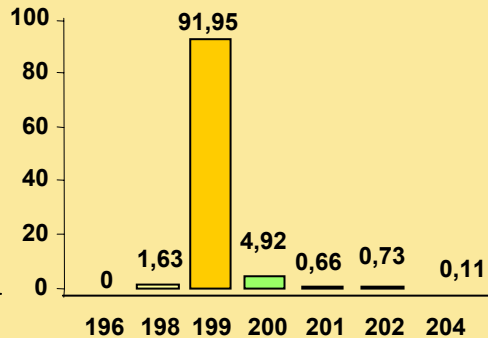


GC-Species Specific Isotope Dilution-ICP-MS

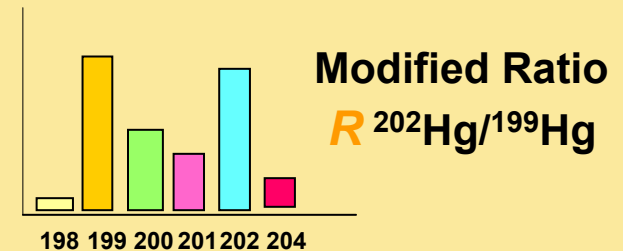
Natural Abundances



Isotopically Enriched



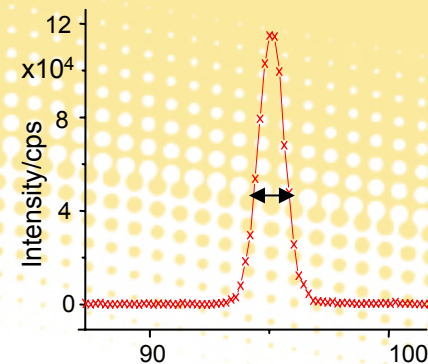
Sample + Isotopically Enriched Standard



$$C = \frac{c'w'A_r(RY' - X')}{wA_r'(X - RY)}$$

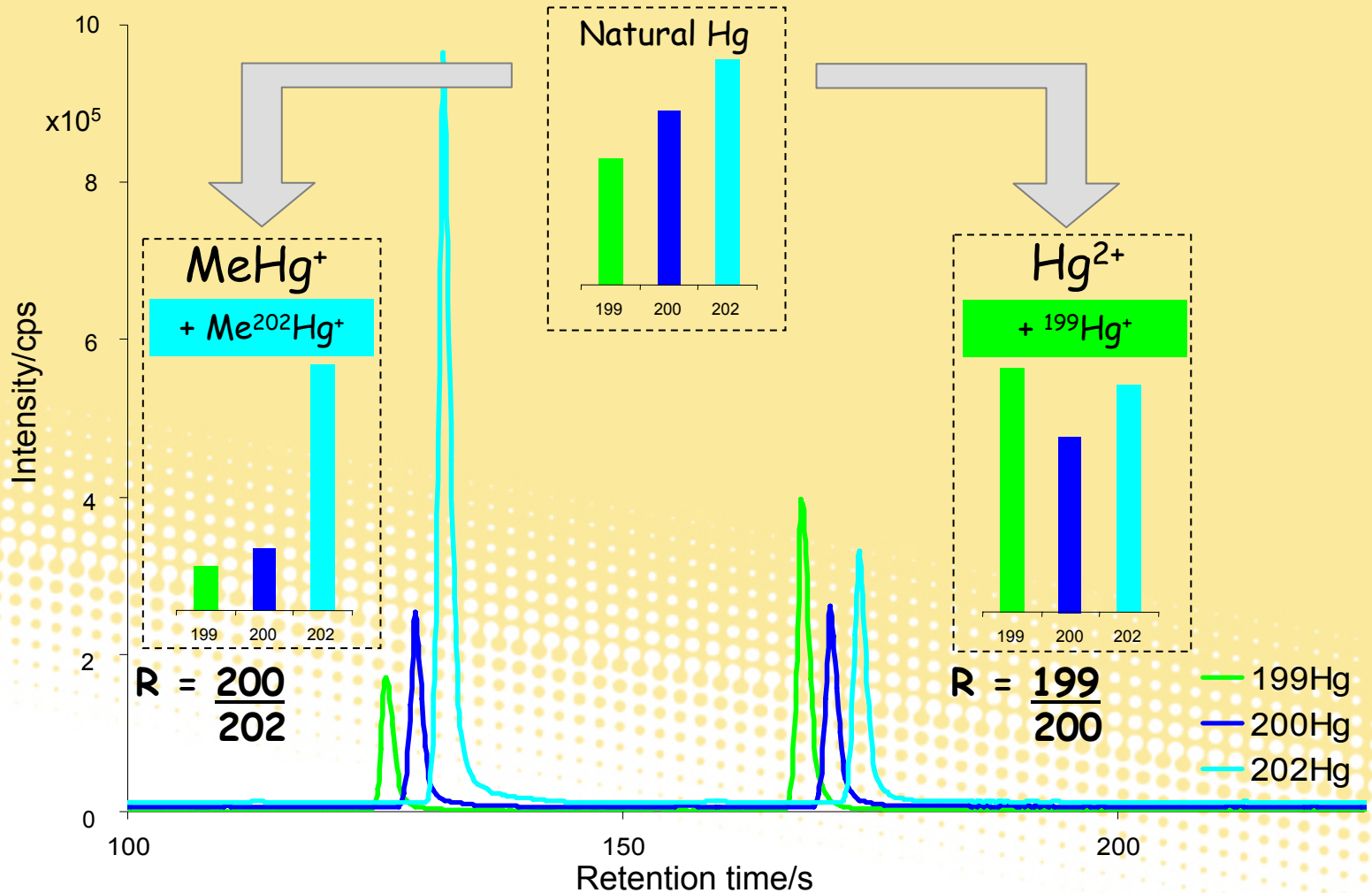
Conditions Required for Species Specific Isotope Dilution

- 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'





1. GC-ID-ICP-MS
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2. HPLC-ICP-MS

Determination of MeHg^+ in Sediments by GC-ID-ICP-MS

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

| CRM | MeHg^+ (ng/g) | Hg^{2+} (ng/g) |
|----------------------------|------------------------|-------------------------|
| BCR 580 (Estuarine) | 75.5 | 132 000 |
| IAEA 356 (Polluted Marine) | 5.87 | 7620 |
| IAEA 405 (Estuarine) | 5.9 | 810 |

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

Determination of MeHg⁺ by SIDMS in Sediments

Sample preparation

Spike-extraction procedure

sediment + spike ²⁰¹MMeHg

+2 ml methanol

Agitation
12H

Dry under
N₂ 3H

+10 ml HNO₃ 6N

Microwave
2min 40W

Direct derivatization

Ethylation

Ethylation in diluted conditions

Solvent
extraction
CH₂Cl₂

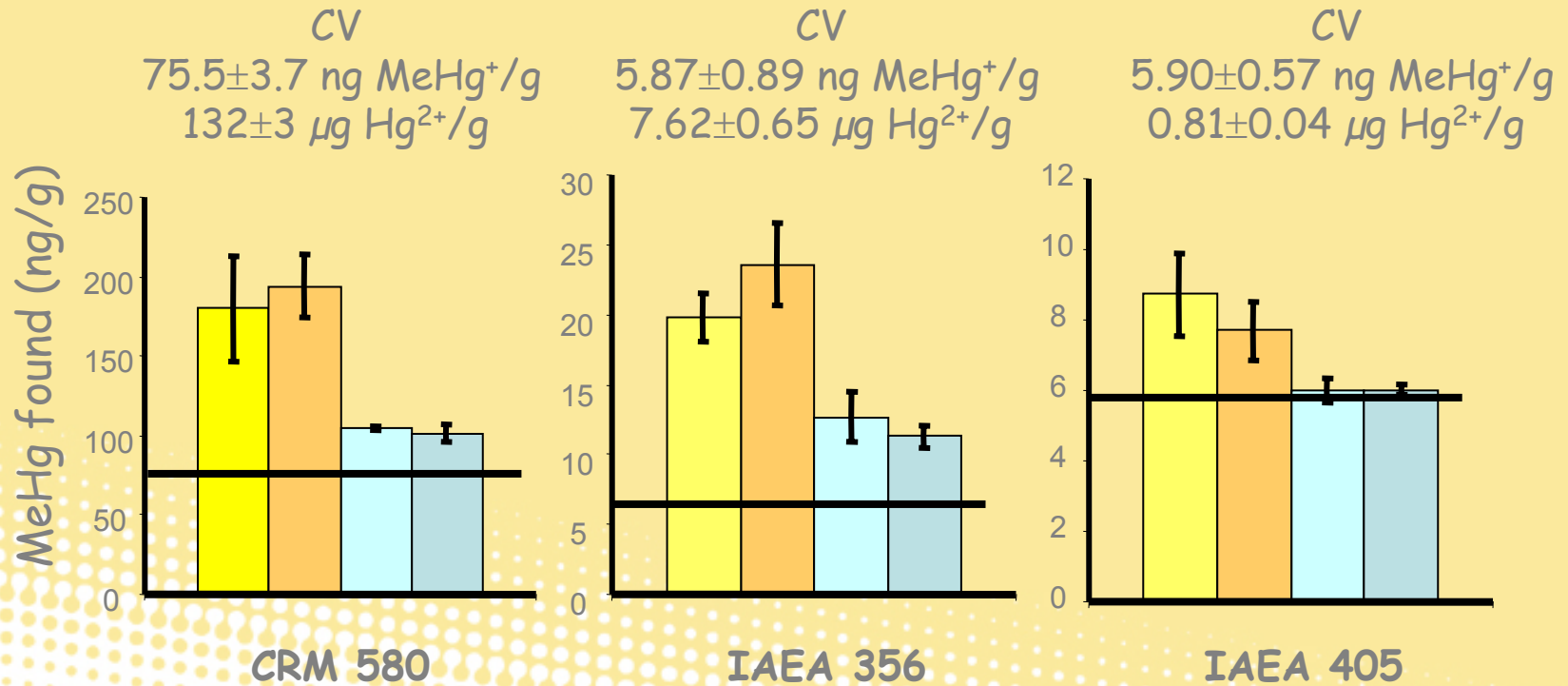
Derivatization

Ethylation

Grignard

Determination of MeHg by SIDMS in Sediments

Application to CRMs



Direct derivatization

- Ethylation
- Ethylation in diluted conditions

Derivatization after CH₂Cl₂ extraction

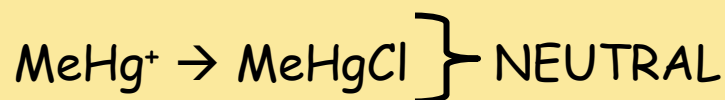
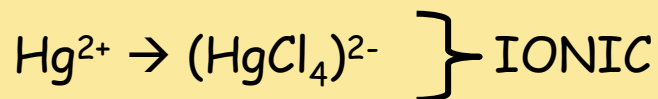
- Ethylation
- Grignard

Determination of MeHg by SIDMS in Sediments

Sample Prep Protocol

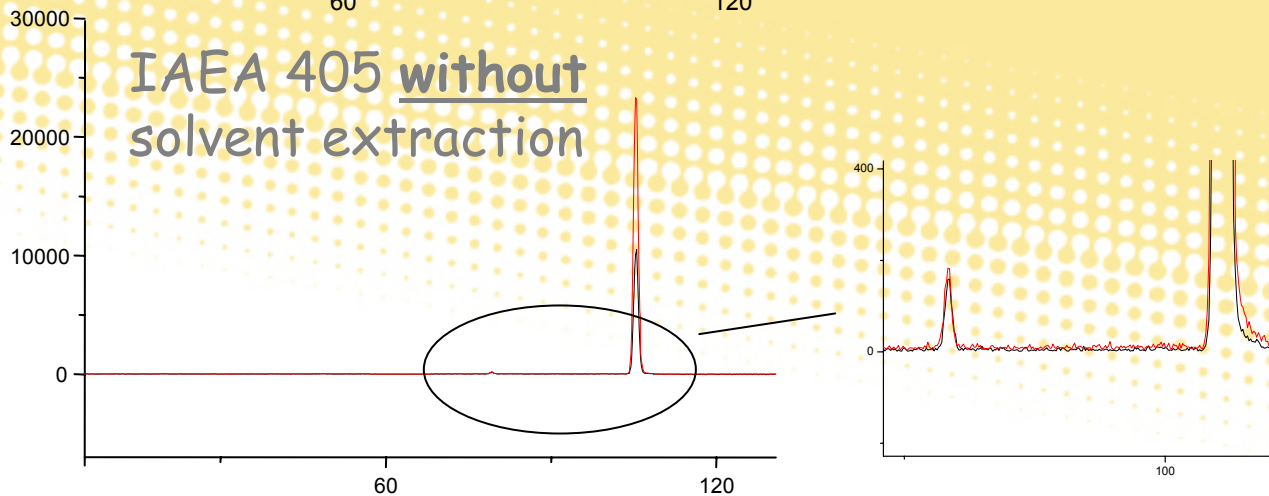
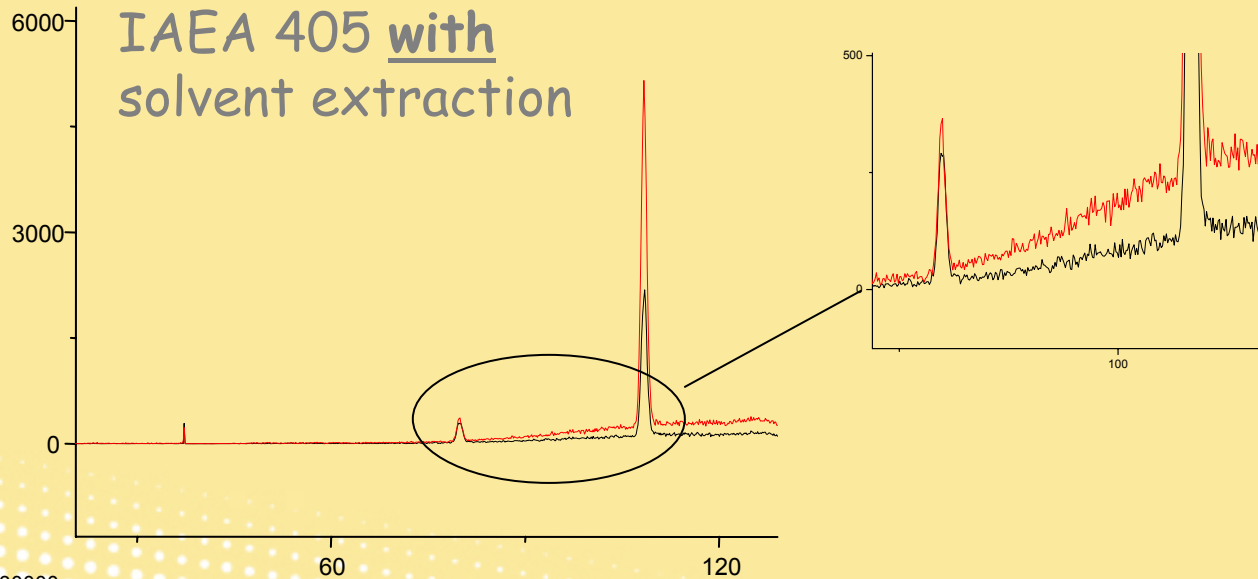
Prevent Artefact Formation:
Extract with CH₂Cl₂ + HCl, 10 mins

Addition of HCl:



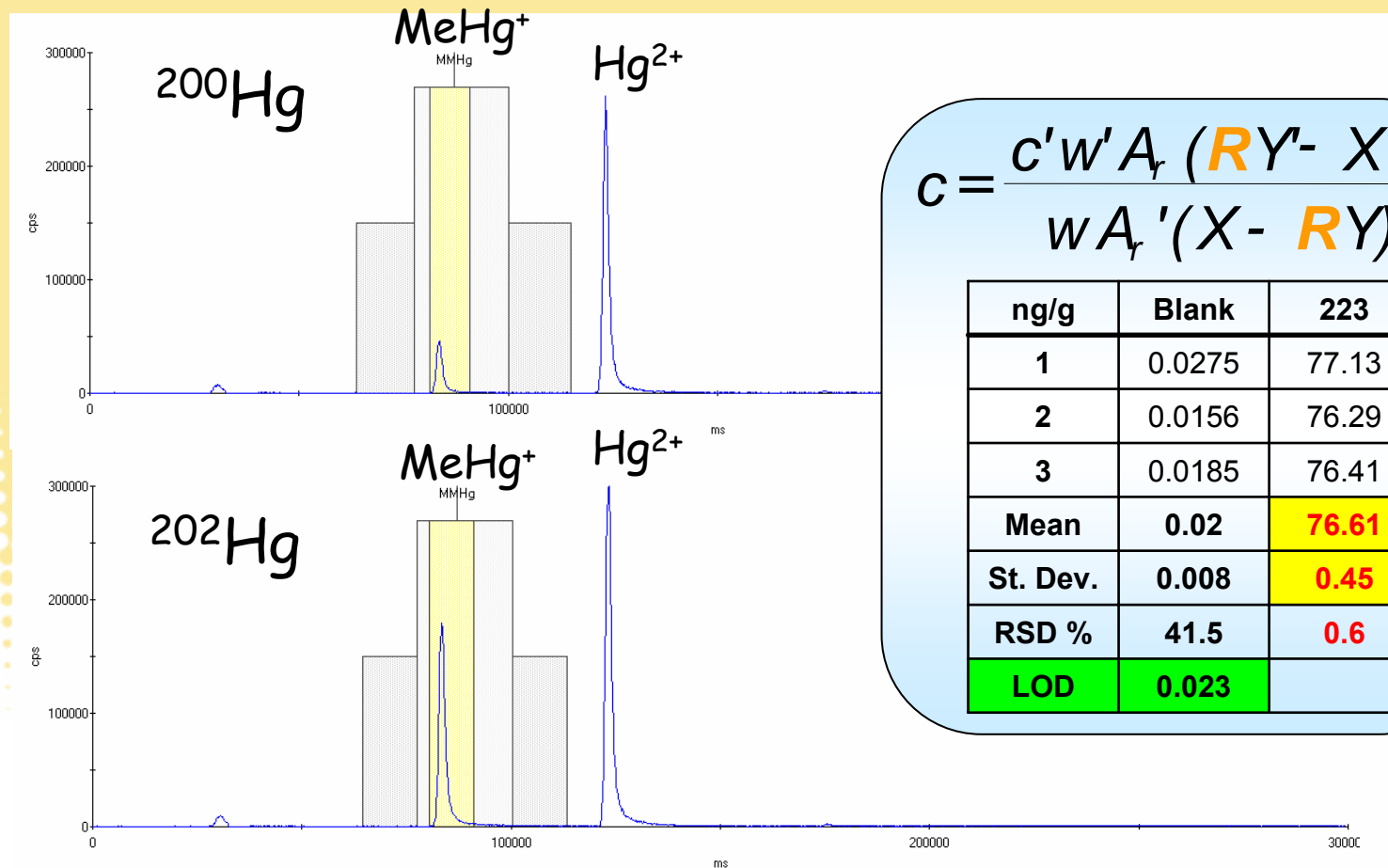
- weigh 1 g sediment
- **add aliquot of isotopically enriched species**
 - **(MeHg⁺ enriched with ²⁰²Hg and Hg²⁺ enriched with ¹⁹⁹Hg)**
- add 5 mL nitric acid (6 N)
- microwave extraction for 3 min at 45 W
- **2 mL extract + 1.5 mL CH₂Cl₂ + 100 μL HCl, shake for 10 min**
- transfer 1 mL aliquot of solvent 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 CH_2Cl_2 Extraction on Speciation of Mercury in Sediments



Determination of MeHg⁺ in Sediments by GC-ID-ICP-MS

BCR 580 with solvent extraction



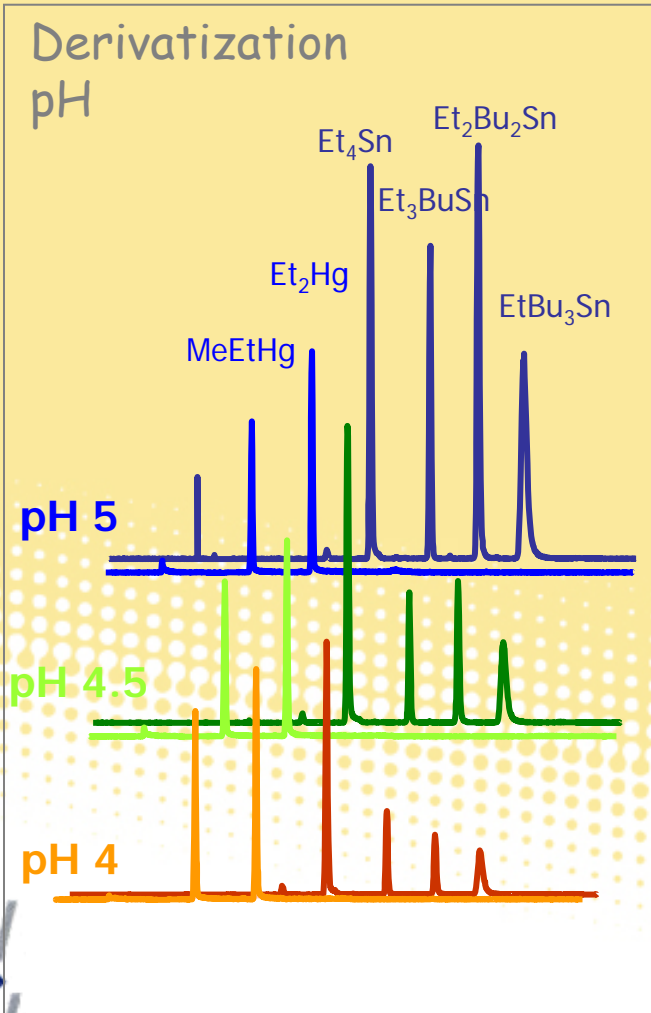
$$C = \frac{c'w'A_r (RY' - X')}{wA_r'(X - RY)}$$

| ng/g | Blank | 223 |
|----------|--------|-------|
| 1 | 0.0275 | 77.13 |
| 2 | 0.0156 | 76.29 |
| 3 | 0.0185 | 76.41 |
| Mean | 0.02 | 76.61 |
| St. Dev. | 0.008 | 0.45 |
| RSD % | 41.5 | 0.6 |
| LOD | 0.023 | |



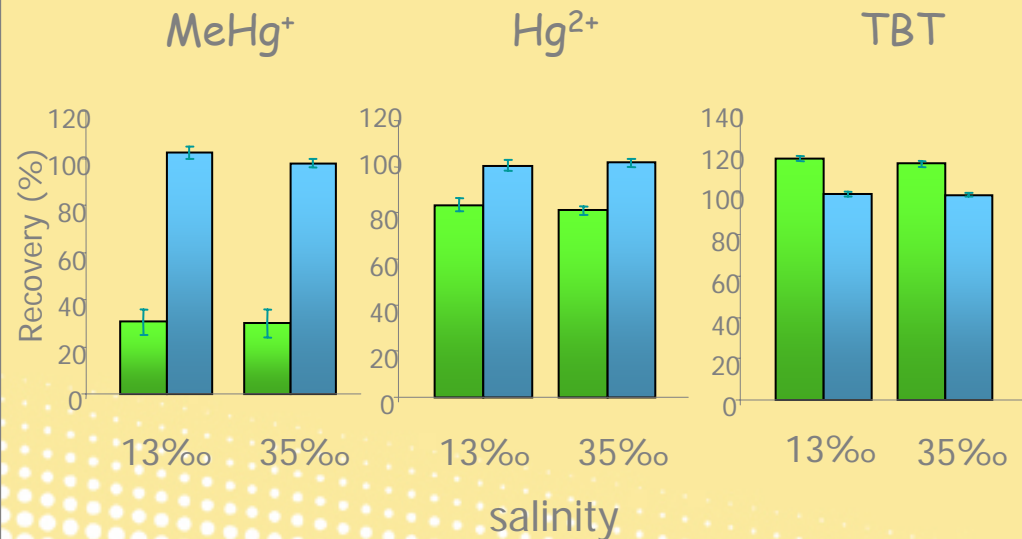
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Simultaneous Mercury and Tin Speciation Optimization of the Sample Prep



Salt Effect

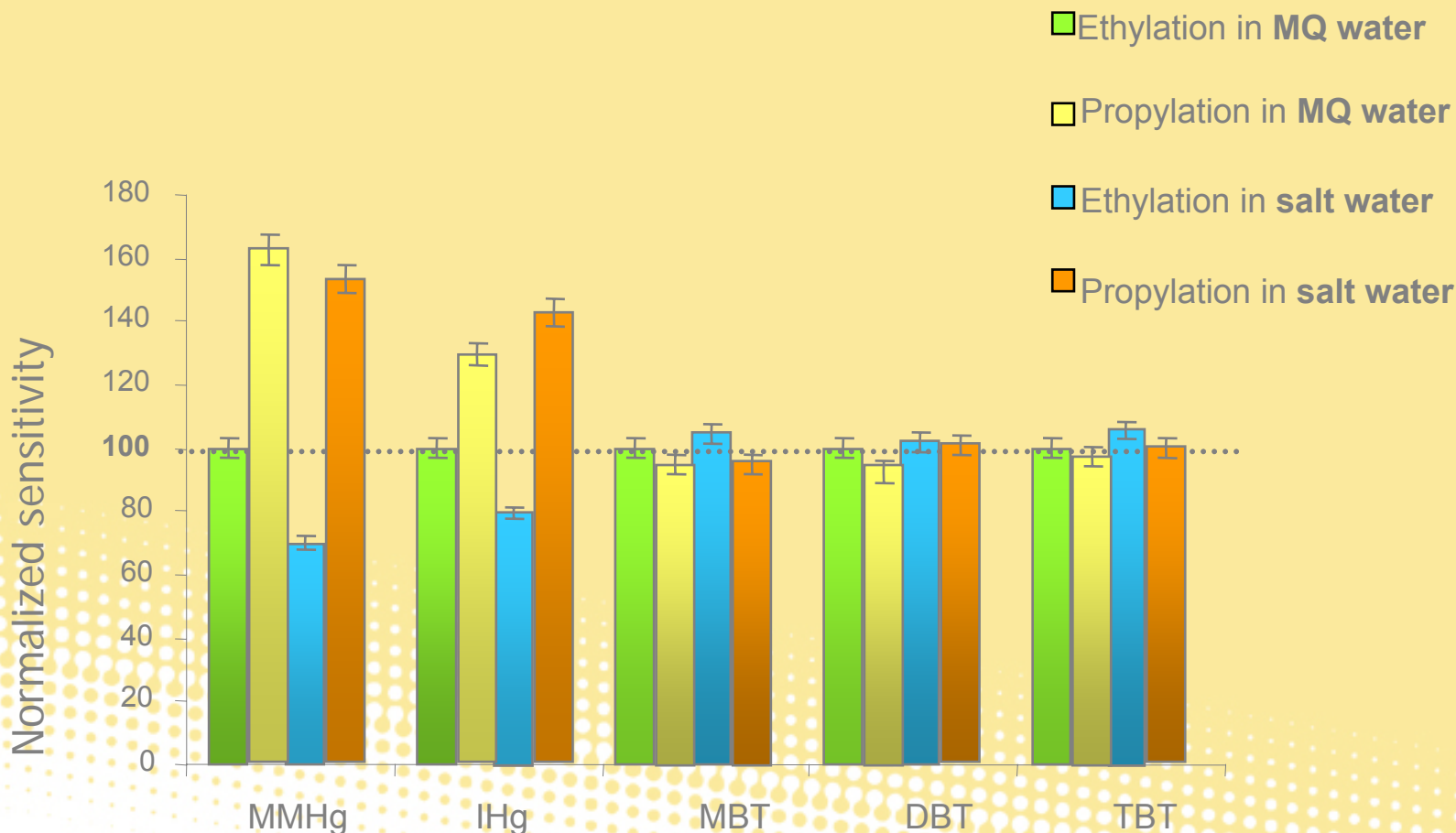
Synthetic sea water spiked with 1 ng/L



- External calibration
- Species specific isotope dilution

Simultaneous Mercury and Tin Speciation

Optimization of the Sample Prep - Derivatization Agent



Hg compounds higher sensitivity with propylation

BuSn compounds same sensitivity

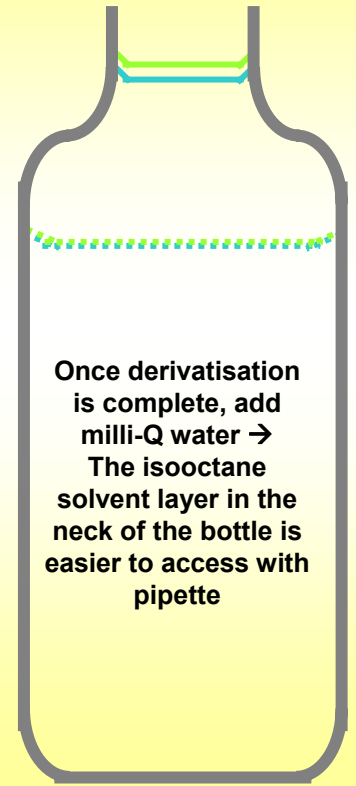
Determination of MeHg^+ by SIDMS in Seawater

Sample Prep Protocol

Preconcentration of Hg Species

- weigh 100 mL water (river, estuarine, sea)
- **add aliquot of isotopically enriched species**
 - **(MeHg^+ enriched with ^{202}Hg and Hg^{2+} enriched with ^{199}Hg)**
- 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% NaBPr_4 and 100 μL - 1 mL isooctane
- shake for 5 min to complete derivatisation
- transfer aliquot of isooctane into GC vial

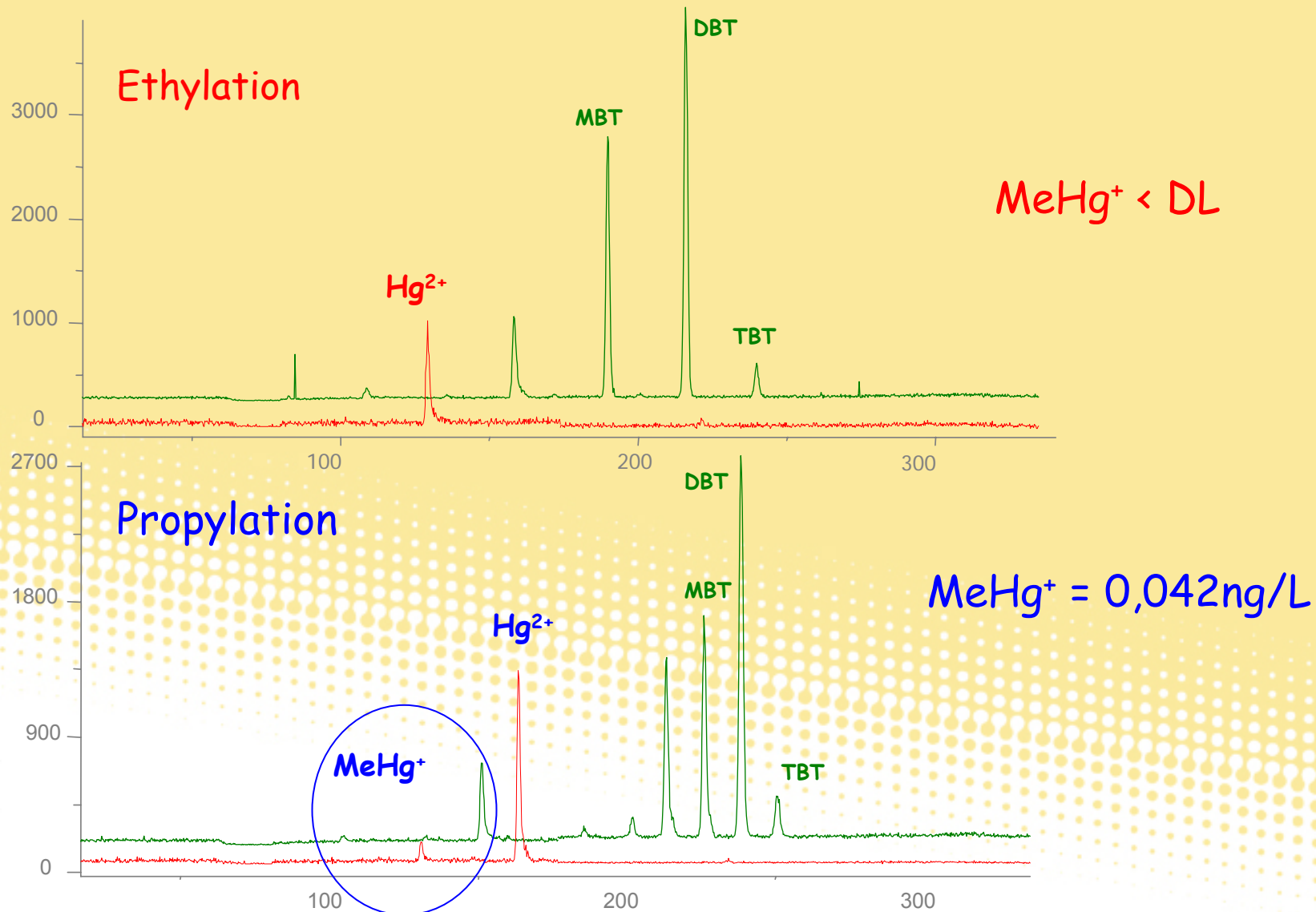
HANDY TIP:



Hg species in 100 mL water → 100 μL isooctane

Simultaneous Mercury and Tin Speciation

Natural Sample of Seawater





1. GC-ID-ICP-MS
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Mercury Speciation Determination of MeHg⁺ in Fish

Sample Prep Protocol

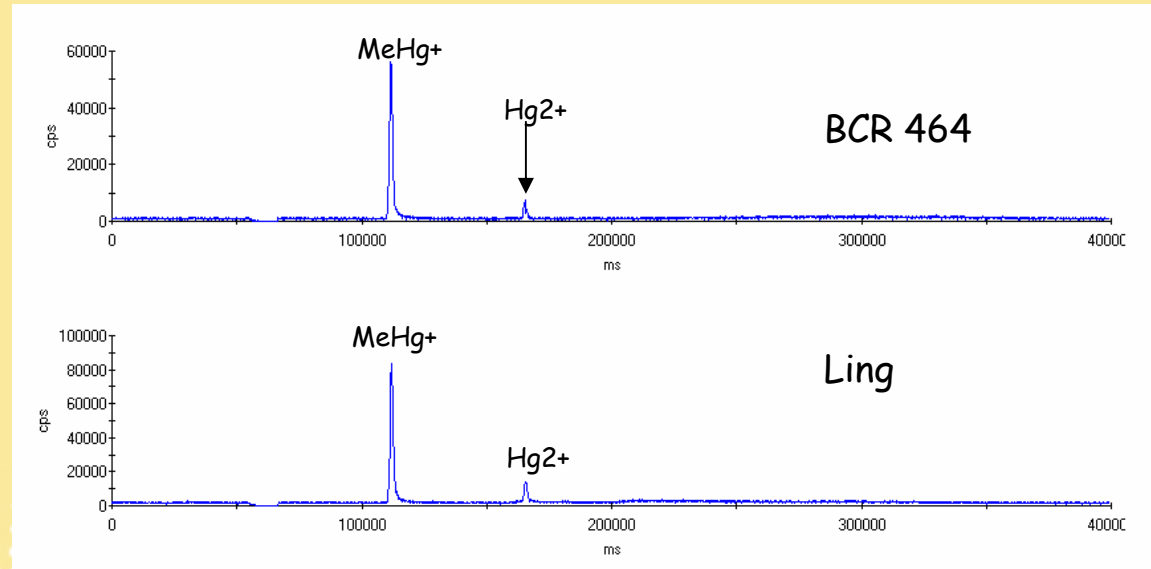


BCR 464 (tuna fish) or fresh fish

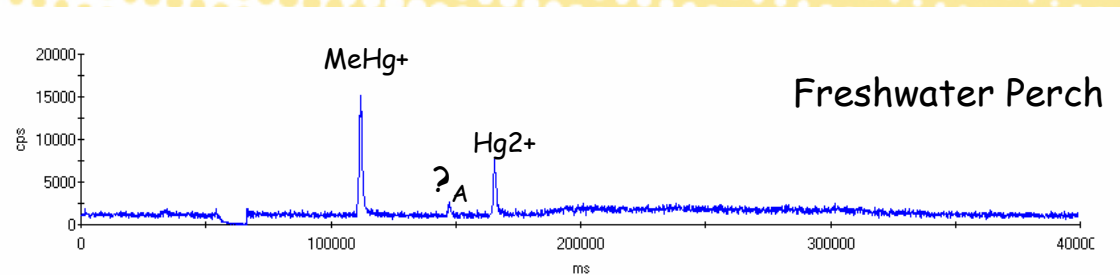
- Freeze dry fresh fish (preservation, ease of handling)
- Grind freeze dried fish tissue with pestle and mortar
- Weigh 250 mg BCR 464 or freeze dried fish
- **add aliquot of isotopically enriched species**
 - **(MeHg⁺ enriched with ²⁰²Hg and Hg²⁺ enriched with ¹⁹⁹Hg)**
- add 5 mL TMAH (25 %)
- microwave 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% NaBEt₄ and 1 mL isooctane
- shake for 5 min to complete derivatisation
- centrifuge and transfer aliquot of isooctane into GC vial

Mercury Speciation Determination of MeHg^+ in Fish

Marine Fish



Freshwater Fish



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

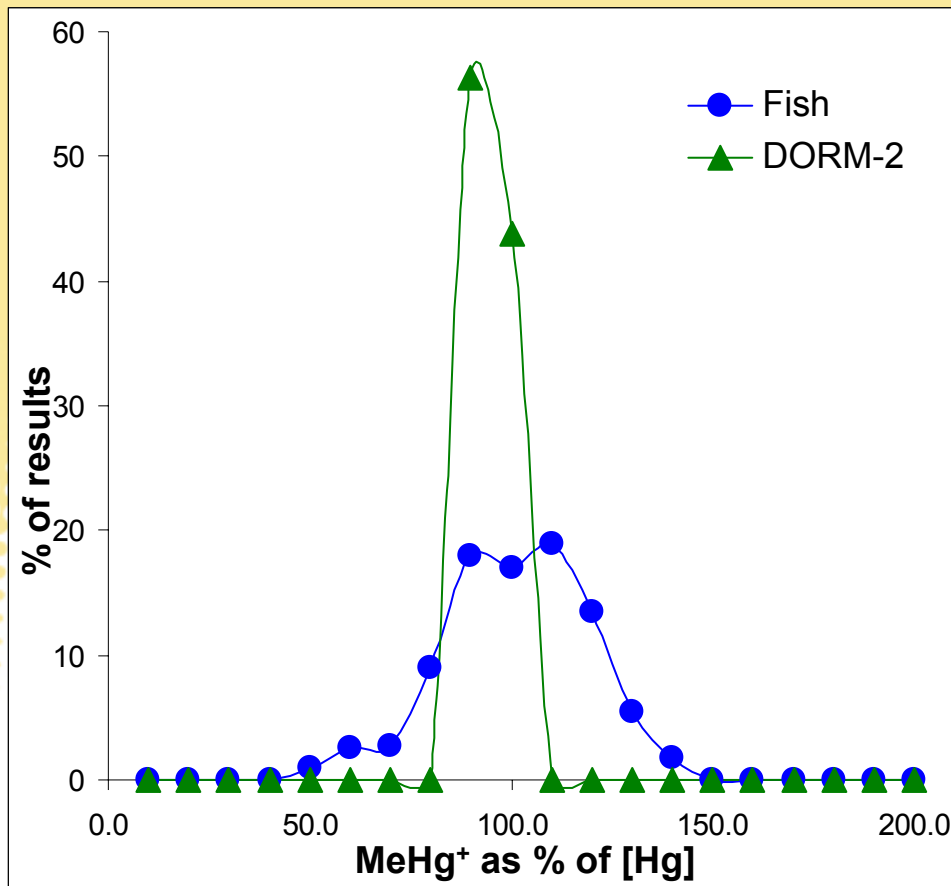
- Derivatisation - adjust pH, propylating agent, isoctane

- GC-ID-ICP-MS

MASS BALANCE for samples where $\text{MeHg}^+ \sim [\text{Hg}]$

Relation Between MeHg⁺ and Total Hg in Commercial Fish Samples and DORM-2

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



| Sample | % of results |
|--------|--------------|
| Fish | 54 |
| DORM-2 | 100 |

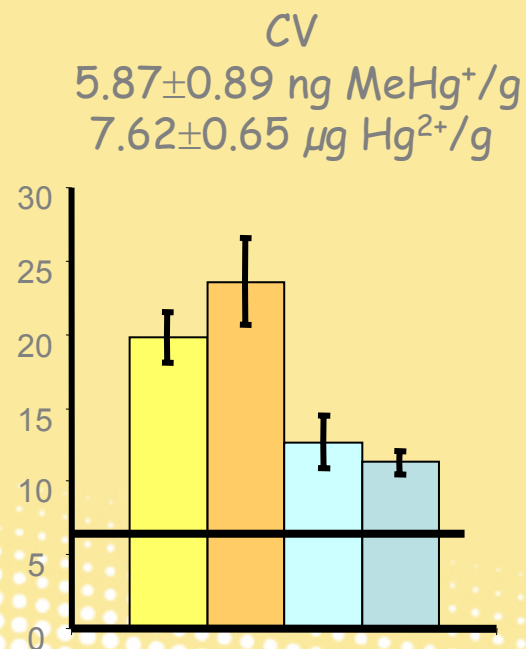
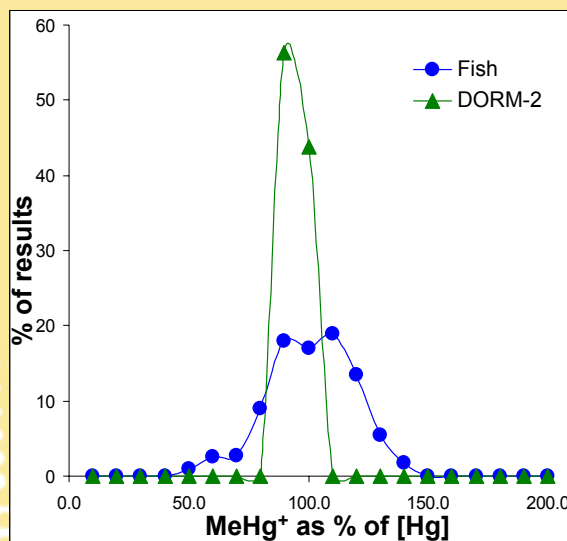
DORM-2 method validation:
[MeHg⁺] = 4.24 ± 0.24 µg/g
(95% of certified value)

**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



IAEA 356

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



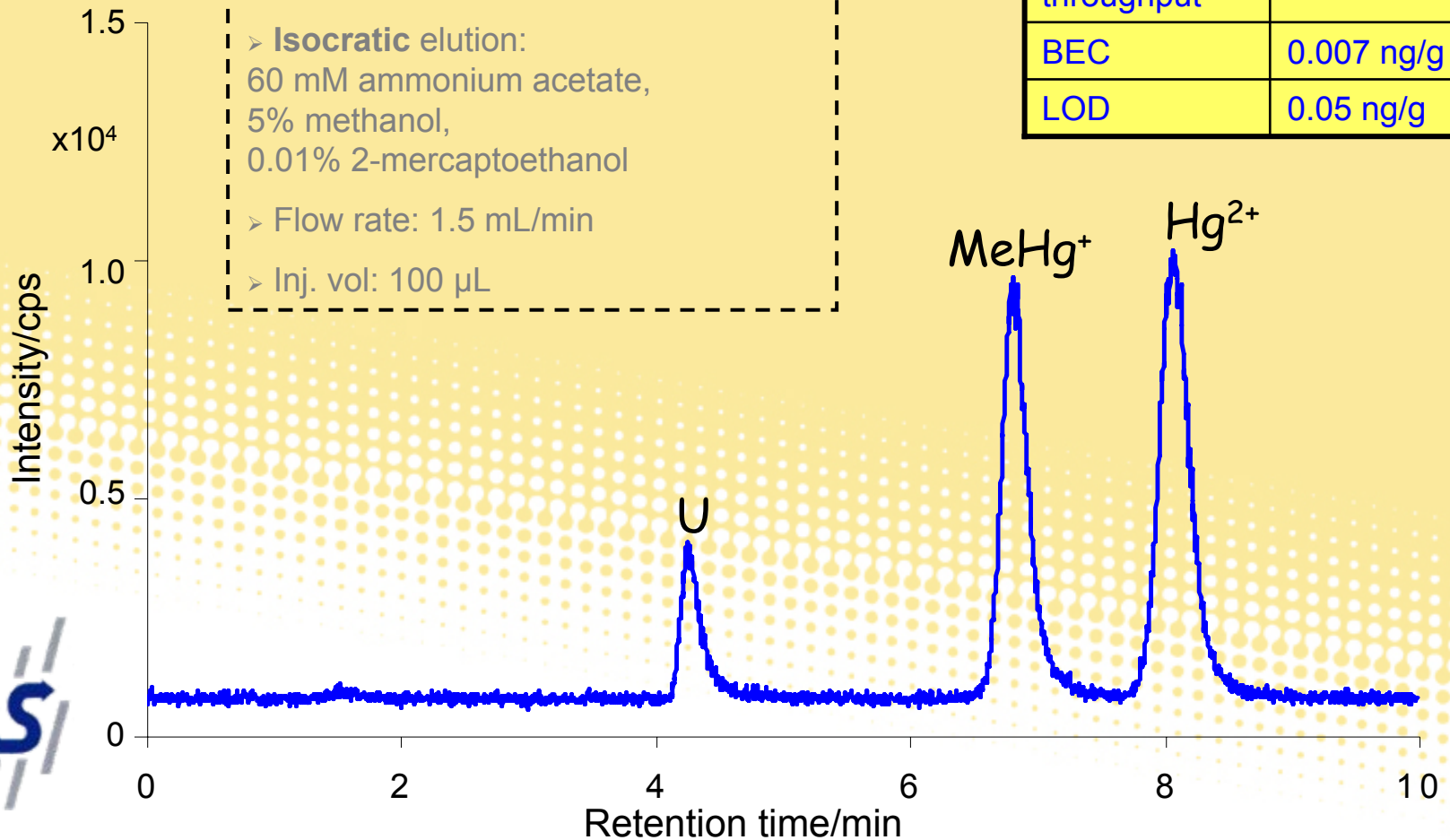
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Speciation of Mercury Reversed Phase HPLC-ICP-MS

HPLC parameters

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

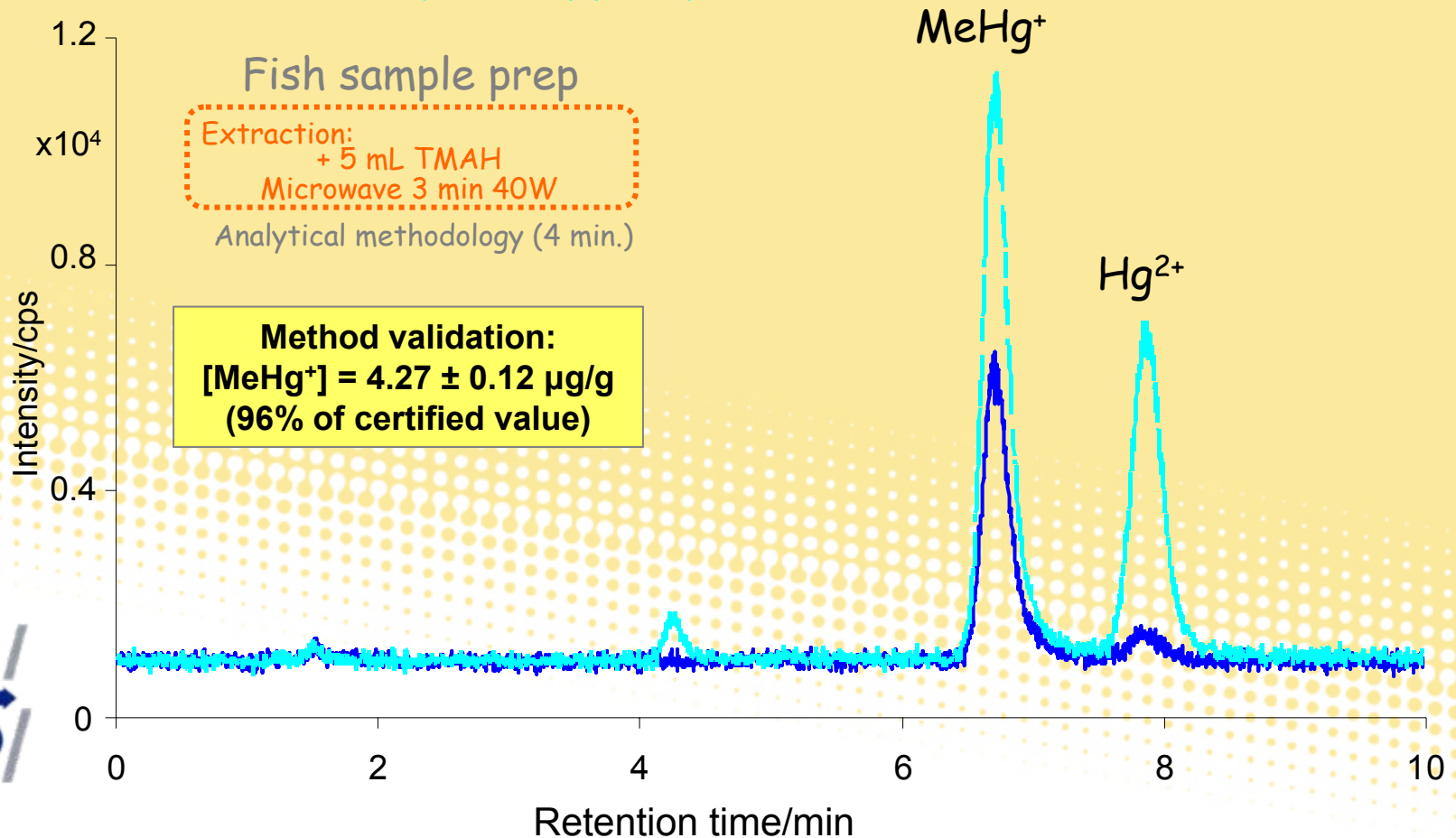
| | |
|-------------------|------------|
| Run time | 10 min |
| Sample throughput | 120/day |
| BEC | 0.007 ng/g |
| LOD | 0.05 ng/g |



Speciation of Mercury in Biological Tissues

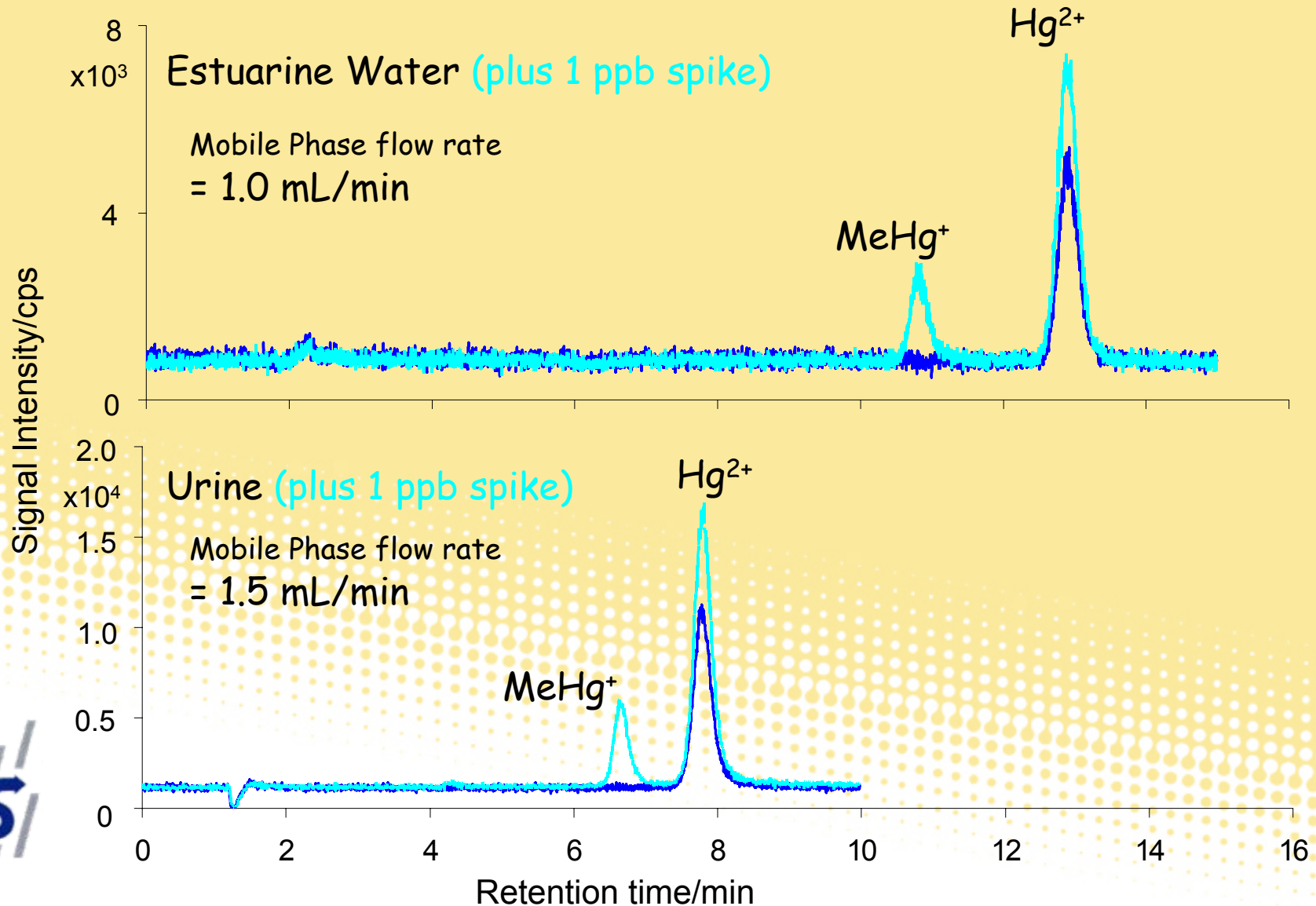
Validation of Technique with CRM

DORM-2 (plus 2 ppb spike)



Speciation of Mercury in Liquid Samples

No Sample Preparation Needed



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

Does Routine Hg Speciation Exist Yet?



Method development and validation with CRMs
Alternative analytical approaches

THANKS !!!!

UT2A

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