# Preparative LC for Hg isotope ratio measurements of Hg species in fish by MC-ICPMS

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Science
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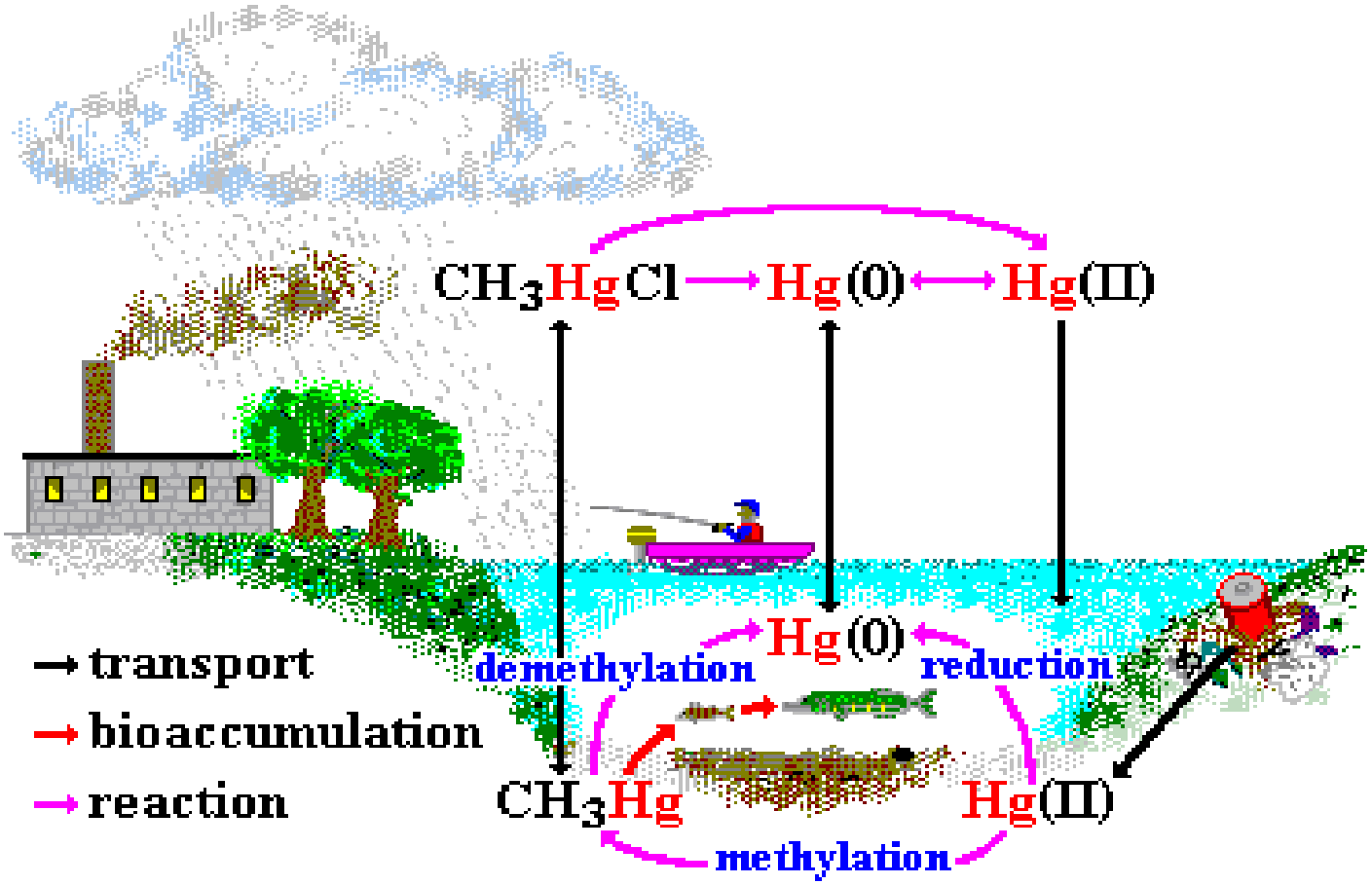
MeTra
Traceability for Mercury Measurements

# Mercury cycle in the biosphere



# Background

- "Minamata Convention on Mercury" A global and legally binding convention adopted at the UNEP Diplomatic Conference held in October 2013 in Japan.
- A Global Mercury Observation System was set up to monitor levels around the world.
- Hg isotopic signature information of the species can provide extra information on biotransformation and sources.

The project -Joint Research Project of the European Metrology Research Programme (ENV51 – MeTra)
Work package 3 : Traceability for mercury isotopic measurements

- **http://projects.lne.eu/JRP/MeTra/project-overview/index.asp**

# History of LGC's contribution to accurate mercury speciation measurement

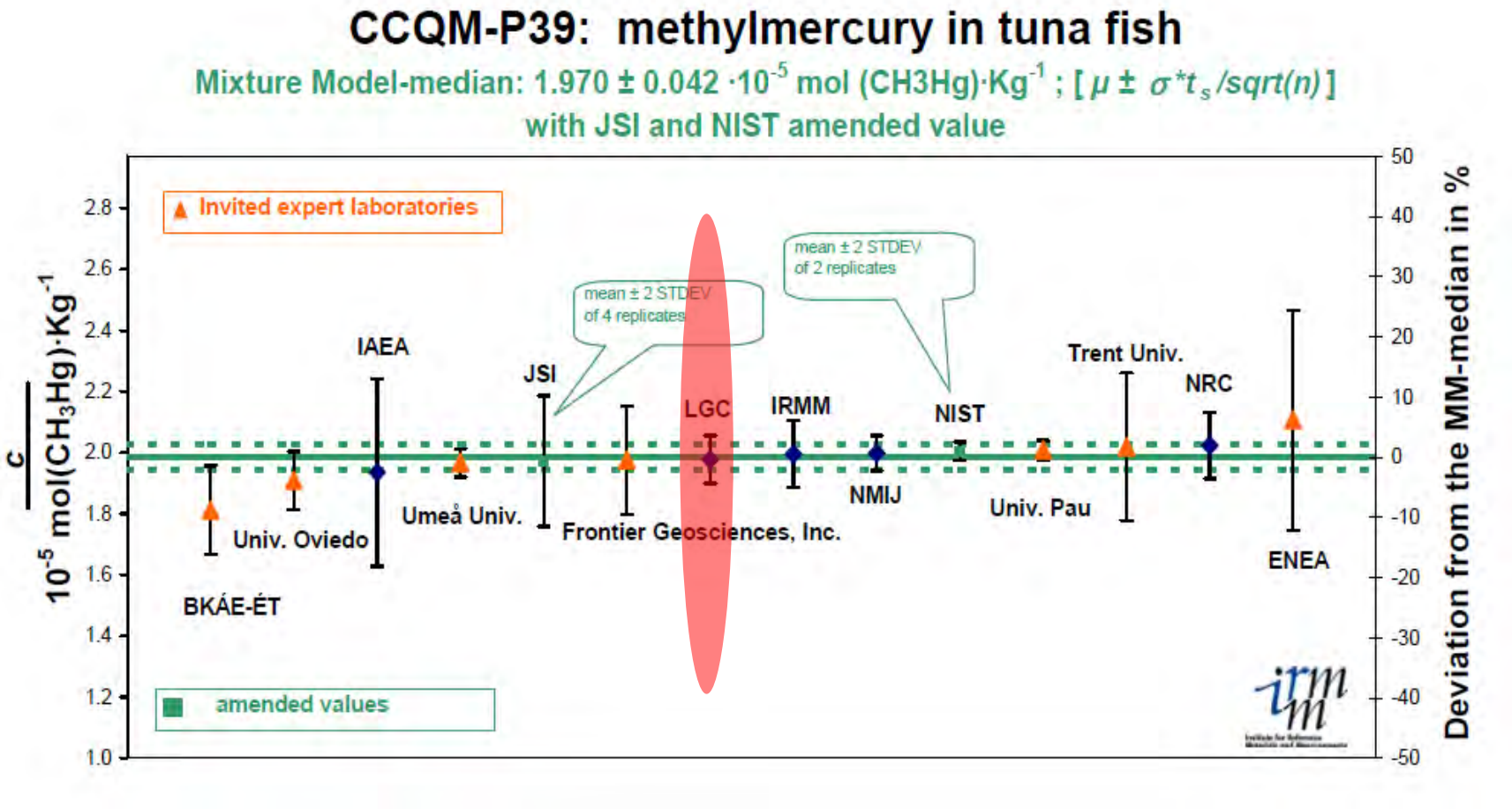
In 1997 Chris Harrington et al published a paper

"Problems Encountered During the Development of a Method for the Speciation of Mercury and Methylmercury by High Performance Liquid Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry"

*J. Anal. At. Spectrom.* 12, 1997, 1053-1056

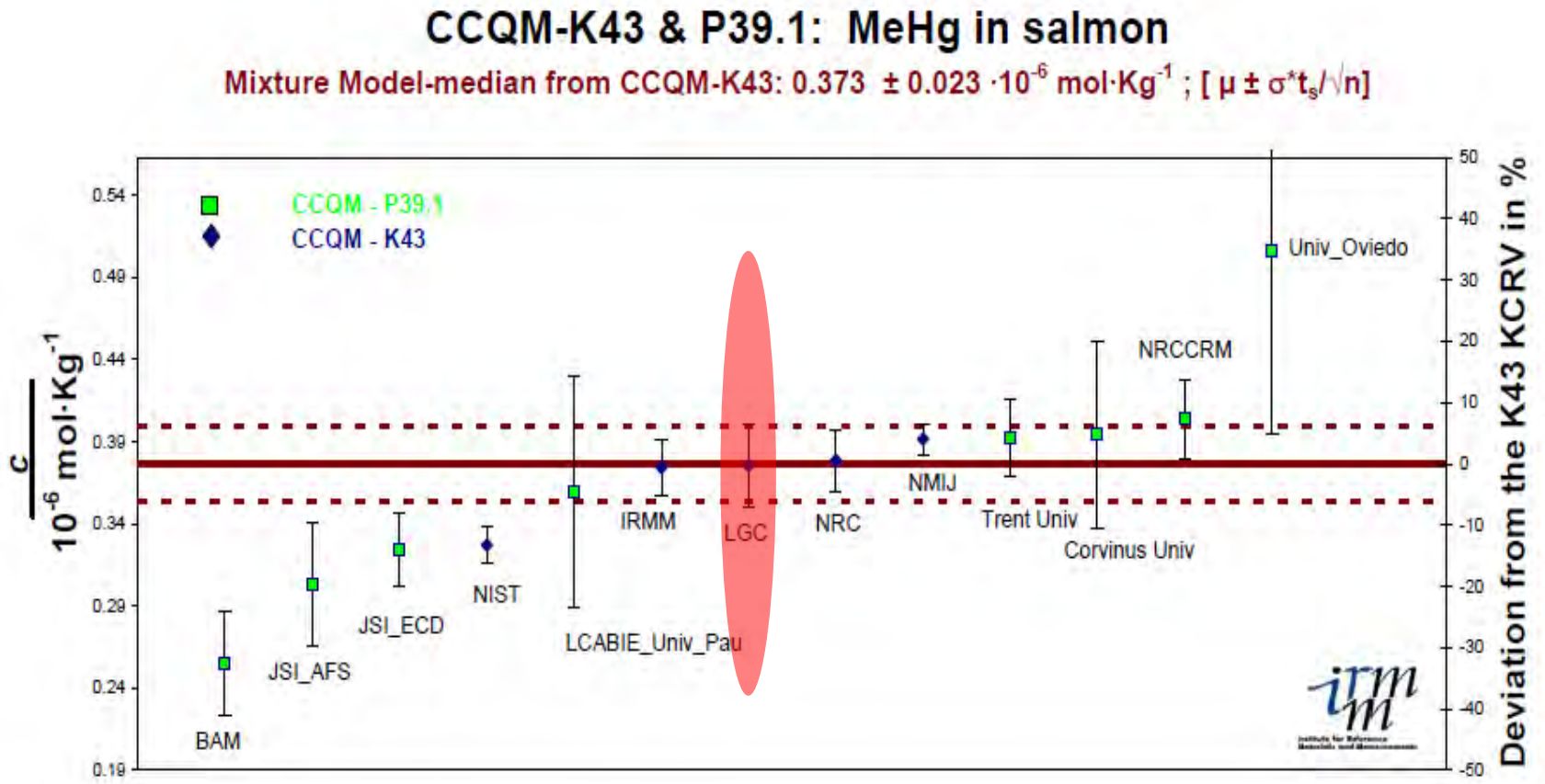
Solution - 2-mercaptoethanol was used to overcome poor peak shape and LC tailing problems.

# 2003 - accurate quantitation of Methylmercury in tuna (defatted fish)




http://www.bipm.org/utils/common/pdf/final_reports/QM/P39/CCQM-P39.pdf

# 2005- accurate quantitation of Methylmercury in salmon (oily fish)




http://www.bipm.org/utils/common/pdf/final_reports/QM/K43/CCQM-K43.pdf

# Development of the procedure for Hg isotope ratio measurements of Hg species

## Steps in the analytical process

- Extraction.
- LC separation.
- Isolation and collection.
- Minimise dilution and concentrate extract.
- Measurement of isotope ratio using multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) (20ng $g^{-1}$ minimum concentration)

# Fractionation of Hg isotopes

- Mercury has 7 naturally occurring isotopes
- In nature the maximum isotopic variability is in the order of 0.8%

| Mass Number | Natural Abundance |
|---|---|
| 196 | 0.15% |
| 198 | 9.97% |
| 199 | 16.87% |
| 200 | 23.10% |
| 201 | 13.18% |
| 202 | 29.86% |
| 204 | 6.87% |

# Selection of sulfhydryl group complexing agent used during both extraction and LC.

2-mercaptoethanol

Advantage

- Stable

Disadvantage

- $Hg^{2+}$ eluted in tail of larger $CH_3Hg$ with low organic modified
- Volatile
- Toxic (bad odour)

Cysteine

Advantage

- $Hg^{2+}$ eluted before larger **$CH_3Hg$**.
- Non-volatile
- Non toxic

Disadvantage

- Easily oxidises

# Extraction

## Challenges

- Quantitative extraction of all species.
- Possible transformation of the species ($CH_3$-Hg $\leftrightarrow$ $Hg^{2+}$).
- Need to minimise dilution.
- Loss of complexing ability by air oxidation of sulfhydryl group.

# Extraction

## Solutions

- Recoveries in good agreement with certified values of CRMs e.g. NIST SRM 1947
- Enzymatic hydrolysis of protein matrix.
- Moderate buffered pH of 7.5 and temperature 37°C.
- Released Hg species stabilised in solution with cysteine.
- Sonication decreases extraction time.
- Minimal sample dilution (1:12) incurred
- Confirmed minimal transformation of species by spiking experiments using labelled species ($^{199}Hg^{2+}$ and methyl$^{202}Hg$).

# LC separation

## Challenges

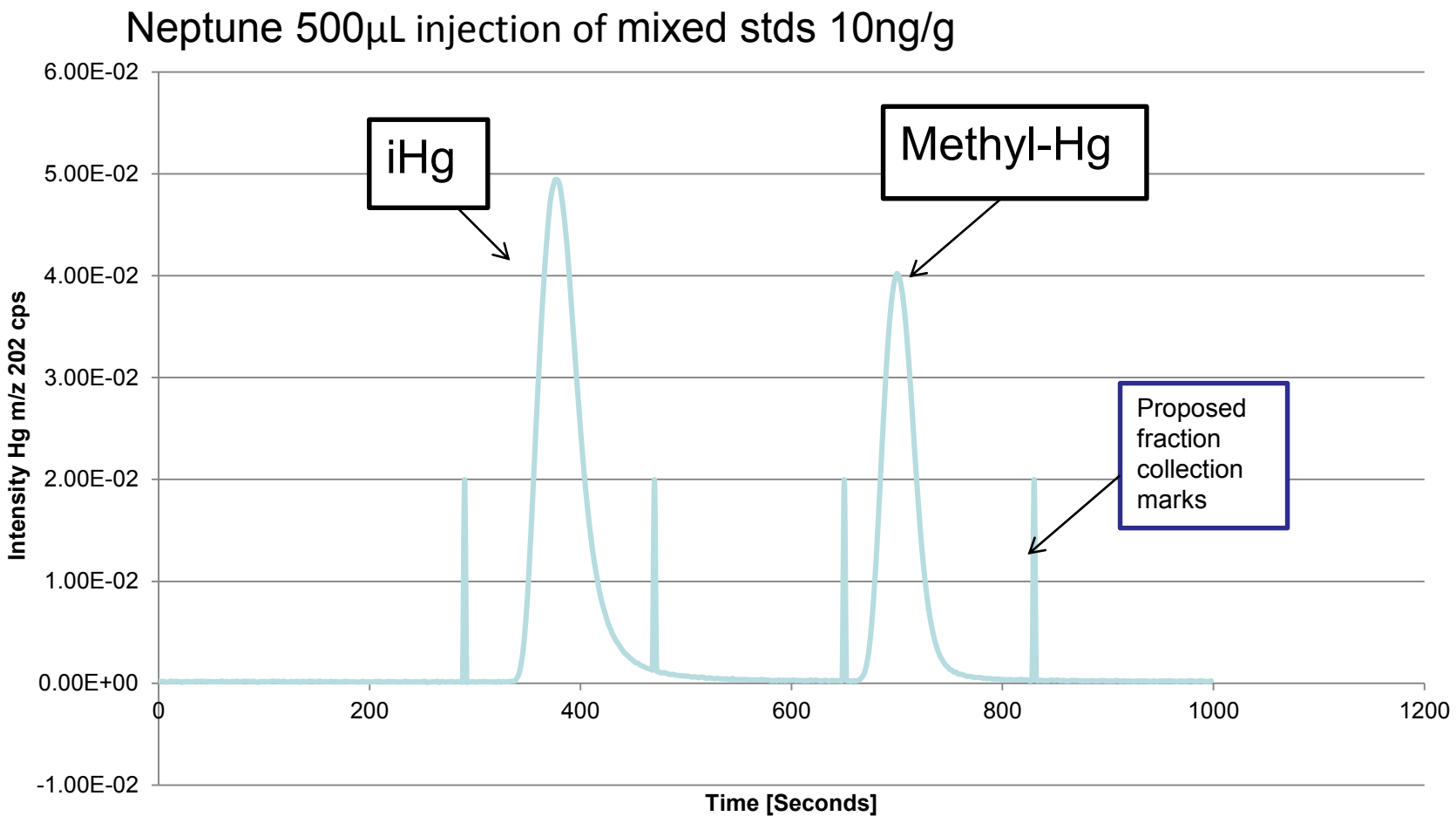
- Require well resolved discrete peaks with minimal tailing.
- Minor species eluted before major species to avoid a collected fraction contain the tailing end of the peak
- Low organic modifier content of mobile phase will ease compatibility with ICP-MS.
- Isocratic is preferred to avoid complexity.
- Capacity for large injection volumes.

# LC separation

## Solutions

- Selection of a reverse phase stationary phase that does not collapses under high aqueous mobile phase.
- Wide bore enables increased loading.
- Selection of cysteine as a complexing reagent as $Hg^{2+}$ eluents first with this reagent.
- Extracts fully compatible with chromatography enabling large injection volumes .
- 100% aqueous buffered phase mobile phase.

# Chromatogram using direct pneumatic nebulisation



# Concentration of collected fractions

## Challenges

- Losses
- Sufficient concentration factor can be obtained.

## Solutions

- Multiple fractions can be collected and combined.
- Freeze drying of collected fraction.
- Use cold vapour [$Hg^{2+} \rightarrow Hg^{0}$] increases signal intensity.

# Extract compatibility with cold vapour generation

## Challenges

- Methyl mercury will not react to form a volatile species
- Sulfhydryl complexing agent cysteine suppresses vapour generation.

## Solutions

- Microwave digestion with nitric acid (Methylmercury → $Hg^{2+)}$
- Cysteine → sulphate which has minimal complexing ability.

# Measurement of isotope ratio analysis

## Challenges

- Instrumental bias
- Fractionation from cold vapour generation.

## Solutions

- Thallium added on line to correct for instrument bias using known $^{205}Tl/^{203}Tl$ isotope ratio of NIST SRM 997.
- Traceable NIST SRM instrumental fractionation caused by cold vapour generation process.

# Control of blank levels

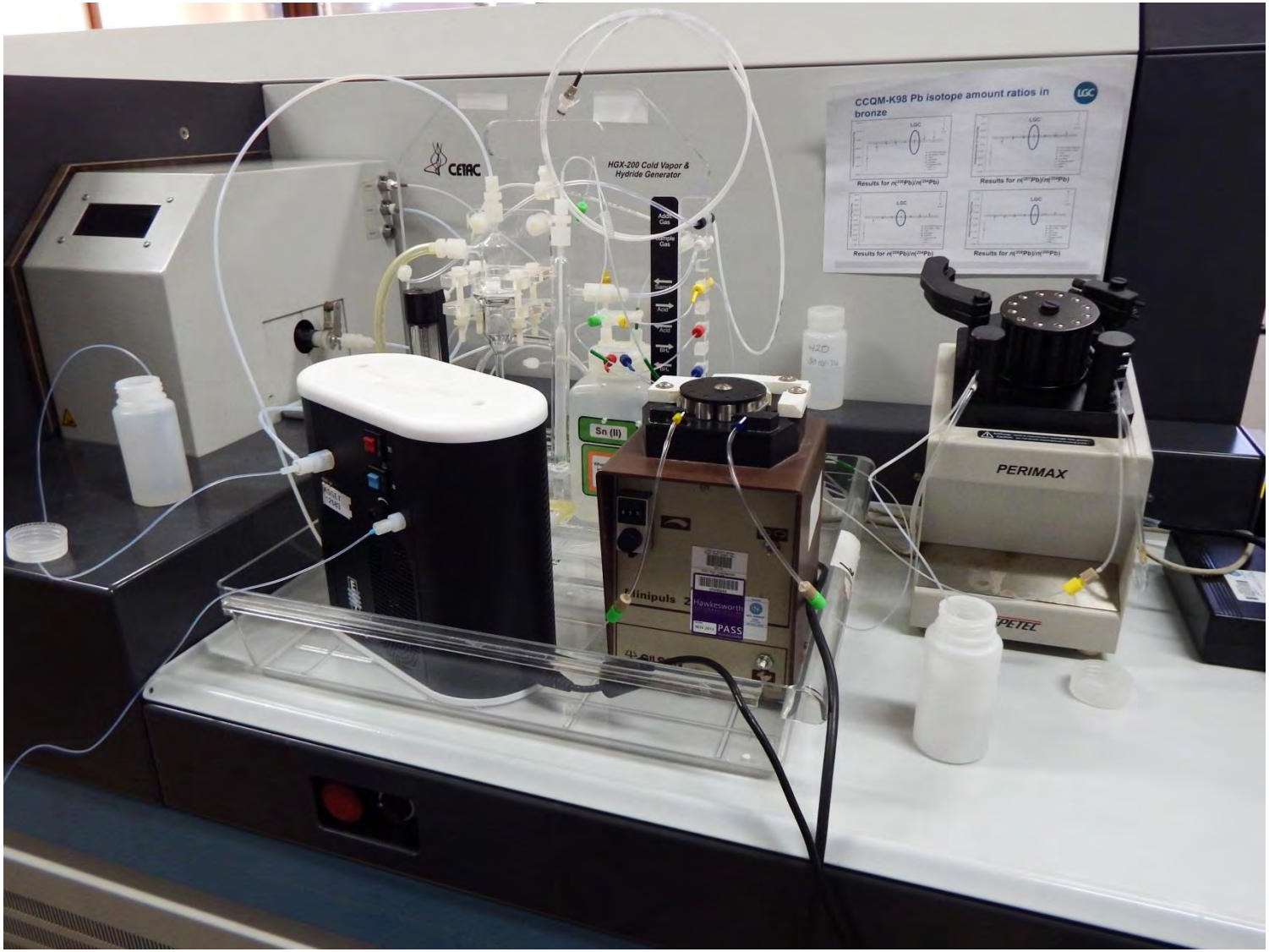
## Challenges

- Reagents
- Equipment
- Laboratory air.

## Solutions

- pH meter probe “Hg free”.
- Enzymes derived from microorganism grown in culture media.
- Minimal exposure to lab air.

# Setup of MC-ICP-MS with cold vapour



# Setup of MC-ICP-MS with cold vapour

# Results of Hg isotope ratio measurements for fish tissues

There is no fish tissue reference material certified for the isotopic composition of mercury, either bulk or species-specific. Below are δ-values for Hg isotope ratios for MeHg in fish tissues reference materials certified for Hg concentration (‰ ± standard deviation at 2σ level).

| | $^{199}Hg/^{198}Hg$ | $^{200}Hg/^{198}Hg$ | $^{201}Hg/^{198}Hg$ | $^{202}Hg/^{198}Hg$ |
|---|---|---|---|---|
| BCR 463 | 1.9 ± 0.2 | 0.3 ± 0.1 | 1.9 ± 0.2 | 0.5 ± 0.1 |
| | | | | |
| NIST 1947 | 4.9 ± 0.2 | 0.5 ± 0.1 | 4.4 ± 0.2 | 1.0 ± 0.1 |

# Conclusion

- Rapid quantitative extraction technique.
- Integrity of Hg species maintained as minimal interconversion incurred.
- $Hg^{2+}$ blank under control.
- No evidence of isotopic fractionation observed.
- Internationally recognised delta value obtained for samples referenced to NIST SRM 3133

# Next step

- Apply the procedure to a range of fish types from different locations.
- More precise collect of fractions less concentrating is required.
- Collected more fractions form a sample digest will increase Hg content of  final extract resulting in better data.
- Isotopic analysis of minor $Hg^{2+}$ species.

# Acknowledgments


- Panayot Petrov
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# Questions?