TEQIP SUMMER INTERNSHIP 2018

RESEARCH TOPIC: Estimation of methyl mercury in marine species using Direct Mercury Analyzer (DMA-80).

INTRODUCTION: The chemical speciation of Hg determines its mobility and toxicity in flooded soils and sediments where in microbial methylation can occur. Bio accumulative (mono)methylmercury (MeHg; CH_3Hg^+) can affect human health particularly via the consumption of contaminated fish and other seafood since MeHg is a potent neurotoxin.

PRINCIPLE OF ANALYSIS: As a positively charged ion, it readily combines with anions and has very high affinity for sulfur containing anions, particularly the thiol (-SH) groups on the amino acid cysteine andhence proteins containing cysteine form a covalent bond.This standard operating procedure describes the analysis of MeHg based on a double liquid-liquid extraction, firstly with organic solvent and subsequentlywith a cysteine solution [1].
The instrumental analysis is performed using DMA-80.An analytical method for simple and rapid determination of MeHg in organism samples were described by Maggi et al. [2]. The proposed method employs the oxygen combustion-gold amalgamation using DMA-80 after complete removal of MeHg by organic extraction and back extraction to an aqueous medium.
MATERIALS ANS REQUIREMENTS:DMA-80, Analytical balance with at least 1 mg of resolution, Centrifuge and Micropipette.
NOTE: All material (vessels, centrifuge tubes, etc.) used was decontaminated with the following procedure: washing with a common detergent rinsing with type I water (three times) and soaking into a clean diluted HNO3 20% (v/v) bath for 24 h at 25 ◦C. Each soaking was followed by an intensive rising with type I water. Finally, all material was dried in clean environment [2].
Reagents and Standards: HBr (preparation), Toluene, L-cysteine, Purified water type I (ASTM).

Instrumentation: The analysis were carried out with a Direct Mercury Analyser (DMA-80, Milestone srl, Italy). The sample (liquid material for MeHg analysis and solid material for T-Hg determination) is dried at 60°C and then thermally decomposed by controlled heating. Decomposition products are carried to a catalyst by an oxygen flow, then sample oxidation is completed and halogens and nitrogen/sulphur oxides are trapped. The final decomposition products pass through a mercury amalgamator which collects Hg[0], Elemental Mercury. The Hg amalgamator is heated to 700°C and the Hg[0] is released and quantified.

PROCEDURE.

- **Sample Preparation for MeHg determination**: Soft tissue from fishes are removed, labeled then either freeze dried for 24 hours or dried at 60^0 C for 3 days.
- **Moisture content:** $\frac{\text{wet weight – dry weight}}{\text{Wet weight}} * 100$
- **Hydrolysis:** Sample weighed out in triplicate; each sample was transferred in 50 mL polypropylene tube with screw caps and hydrolyzed HBr.After 5 min of shaking using a mechanical vertical shaker, toluene was added (Fig. 1).
- **Methyl Mercury Extraction:** Toluene is added to the sample. All samples were vigorously mixed for 20 min. After centrifugation (2400 rpm for 20 min) the supernatant, containing organomercury species, was collected in 50 mL polypropylene tube. The whole procedure was repeated: toluene was added again to tube containing marine organisms. The combined organic extracts were subjected twice to back extraction with 1% (v/w) L-cysteine aqueous solution to strip MeHg from toluene. Then, an aliquot of L-cysteine extract was immediately analysed with mercury analyser (DMA-80) [2].

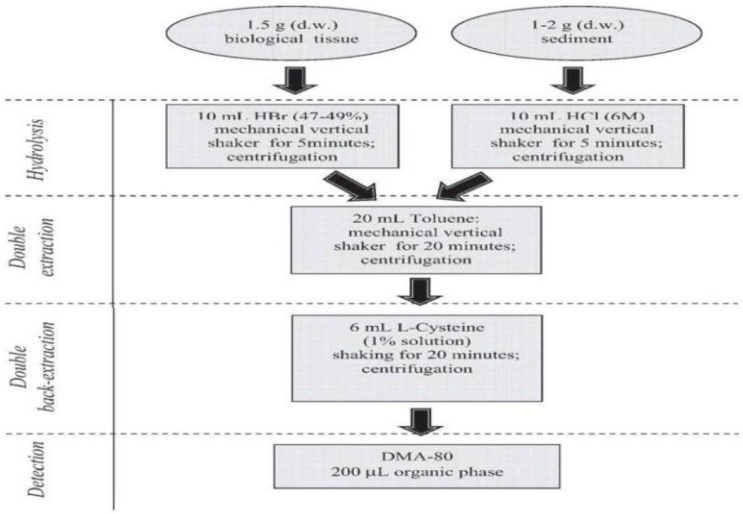

Fig. 1. Scheme of the proposed method for MeHg analysis in sediment and biological tissues.

CALCULATIONS :

$$Concentration\ of\ MeHg\ in\ the\ sample\ \left(\frac{\mu g}{kg}\right) = \frac{Mass\ of\ MeHg\ from\ DMA\ (ng) * Vol.of\ L-cysteine\ (ml)}{Vol.of\ aliquot\ of\ L-cys\ pertaining\ to\ sample\ (ml) * Mass\ of\ sample\ used\ for\ extraction\ (g)}$$

RESULTS:

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Table1. Concentration of MeHg in Tuna and Certified Reference Material (Hair CRM).[3]

Sample	Methyl mercury concentration (µg/kg)
Tuna 1 (by wet weight)	116.19
Tuna 2 (by wet weight)	119.05
Tuna 3 (by wet weight)	119.78
Hair CRM 1	246.95
Hair CRM 2	207.65

CONCLUSION: The analytical performance of the measurements for MeHg concentration for hair CRM ranges in 236 - 279 µg/kg, hence the experiment clears the acceptance criterion.

REFRENCES: [1] Chiara Maggia, Maria Teresa Berduccia, Jessica Bianchia,*, Michele Gianib, Luigi Campanellac

[2] Cordeiro, F., Gonçalves, S., Calderón, J., Robouch, P., Emteborg, H., Connely, P.,& de la Calle, B. (2013). IMEP-115: Determination of methylmercury in seafood. *A collaborative trial report, EUR, 25830*.

[3] Marrugo-Negrete, José, et al. "Total mercury and methylmercury concentrations in fish from the Mojana region of Colombia." *Environmental Geochemistry and Health* 30.1 (2008): 21-30.

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