

Determination of Mercury in Seafood by Atomic Emission Spectrophotometry

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ABSTRACT

Mercury is a known neurotoxin that is particularly harmful to children and unborn fetuses. Consumption of contaminated seafood is one major route of mercury exposure. We have successfully optimized one protocol to determine mercury in seafood products by atomic emission spectrophotometry (AES) with following conditions: speed current NaBH 1% in 6 ml per minute, NaOH 0.5% in 2 ml per minutes, HCl 6M has the same speed current with NaBH4 as the reduced medium, linear range 0.2 - 10ppb, LOD = 0.04ppb, LOQ = 0.15 ppb (CV<10%).

Keywords: Mercury, analysis, seafood, atomic emission spectrophotometry

INTRODUCTION

Seafood is widely consumed in many parts of the world because it has high protein content. Mercury is a dangerous xenobiotic, particularly its vapours and some of its water-soluble salts; one of its properties is the ability to accumulate in the internal organs of living organisms. Mercury enters the food chain with the help of aquatic microorganisms and released into the environment with inorganic form. Mercury is methylated by bacteria in water and converted to an organic form usually methylmercury.

Chwei-Sheng Chiou *et al.*³ determined mercury compounds in fish by microwave-assisted extraction and liquid chromatography-vapor generation-inductively coupled plasma mass spectrometry. A method employing a vapor generation system and LC combined with inductively coupled plasma mass spectrometry LC-ICP-MS is presented for the determination of mercury in biological tissues. An open vessel microwave digestion system was used to extract the mercury compounds from the sample matrix. The efficiency of the mobile phase, a mixture of L-cysteine and 2-mercaptoethanol, was evaluated for LC separation of inorganic mercury Hg II, methylmercury methyl-Hg and ethylmercury ethyl-Hg. The sensitivity, detection limits and repeatability of the liquid chromatography LC ICP-MS system with a vapor generator were comparable to, or better than, that of an LC-ICP-MS system with conventional pneumatic nebulization, or other sample introduction techniques. The experimental detection limits for various mercury species were in the range of 0.050.09 ng ml⁻¹ Hg, based on peak height. The proposed method was successfully applied to the determination of mercury compounds in a swordfish sample purchased from the local market.

A. V. Yallouz, R. Calixto de Campos, S. Paciornik¹³ showed a low-cost non instrumental method for semiquantitative determination of mercury in fish. The sample is acid digested and the mercury vapor released after chemical reduction with SnCl₂. The mercury vapor is then collected on a detecting paper covered with an emulsion of Cu₂I₂, 3% carboxymethylcellulose and MgCl₂ as moistener agent. The colored Cu₂[HgI₄] complex is formed and the color intensity is proportional to the mercury concentration in the original sample.

P. Houserova *et al.*⁶ determined total mercury and mercury species in fish and aquatic ecosystems of Moravian rivers. Five tissues (muscle, gills, liver, kidney and skin) of chub (*Leuciscus cephalus*), zoobenthos, sediments and water samples were analyzed. Time stability of samples was also tested.

The highest levels of total mercury were determined in muscle tissues of all tested fish. Relative contents of MeHg in muscle tissues of fish ranged from 83.6% to 92.0% of the total mercury contents. The relative contents of MeHg in sediments and in zoobenthos samples correlate very closely (correlation coefficient – 0.83). A considerably lower content of MeHg (1.3–11.4%) was found in river sediments compared with lakes. A comparison of observed sampling sites (Vladislav, Hrubšice) proved the adverse effect of industrial contamination on the water ecosystem of Jihlava River and incomplete removal of mercury species in a sewage station.

Ozlem ErdoRul⁸ determined mercury levels in edible tissues of various fish samples from Dam Lake. The fish samples were obtained from fishermen. Hg levels ranged from 0.03 to 0.18 µg/g dry weight in carp samples and from 0.16 to 0.38 µg/g dry weight.

Piotr Konieczka *et al.*¹⁰ described the validation of CV-AAS for determining the total mercury content in biological samples (whole fish, cormorant tissues). The development and optimization of the procedure is outlined, and the main objective of this study was to calculate its validation parameters. The selectivity of the method was documented; linearity ($r > 0.993$) ranged from 0.29 to 100 ng of total mercury per sample mass. For a total Hg content of 80–1,000 ng, a polynomial calibration curve derived directly the Lambert-Beer law was used. The method showed good recoveries (average 98.0%) and a relative standard deviation for repeatability of < 10%. The limit of detection was calculated at 0.096 ng of total Hg per sample mass.

Ekpo, K. E. *et al.*⁵ determined lead, cadmium and mercury in surrounding water and organs of some species of fish from Ikpoba river in Benin city, Nigeria. The mean concentration of some heavy metals (lead, cadmium and mercury) in the muscles and the organs of some common species of fish: *Metacembelus Iconnbergii*, *Clarias lazera*, *Citarinus citharus*, *Tilapia zilli* and *Erpetoichthy* were investigated using atomic absorption spectrophotometric method. The mean concentrations of lead in the muscle, kidney and liver were in the ranges of 0.00 – 0.004 mg/kg, 0.010 – 0.015 mg/kg, and 0.004 – 0.010 mg/kg respectively, while that of the surrounding waters were between and 0.001 – 0.005 mg/kg. Cadmium concentrations were in the range of 0.001 – 0.002 mg/kg in the muscles, 0.004 – 0.006 mg/kg in the kidney and 0.002 – 0.004 mg/kg in the liver while that of the surrounding water was 0.001 mg/kg. The levels of mercury were 0.001– 0.002 mg/kg in the muscle, 0.004 – 0.006 mg/kg in the kidney, 0.002 – 0.004 mg/kg in the liver and 0.001 – 0.002 mg/kg in the surrounding waters.

Maria-Cristina Radulescu and Andrei Florin Danet⁷ studied the electrochemical behavior of such an electrode and the working parameters for mercury determination by chronopotentiometric stripping analysis. Detection limit was 0.30 µg Hg/L and determination limit was 1.0 µg Hg/L for a deposition time of 600 s. Using the developed working electrodes it was possible to determine the total mercury in fish samples. A method for fish sample digestion was developed by using a mixture of fuming nitric acid and both concentrated sulfuric and hydrochloric acids. The recovery degree for a known amount of mercury introduced in the sample before digestion was 95.3% (n=4).

Ebrahim Rahimi and Asma Behzadnia⁴ determined mercury in canned tuna fish produced and distributed in Iran after digestion by the standard methods of AOAC. Mercury contents in fish and canned tuna fish were determined by cold vapor atomic absorption spectrophotometer. The metal content, expressed in mg/kg wet weight for mercury varied from 0.017 to 0.394 (average of 0.089) and 0.023 to 0.529 (average of 0.146) in fish and canned tuna fish, respectively. The values were comparable and in the range of with the literature values. The results of this study indicate that fish and tuna fish of produced and marketed in Iran have concentrations well below the standards FAO/WHO levels of these toxic metals and only one tuna samples exceeded the European dietary limit of 0.5 mg Hg/kg.

J. Salaramoli *et al.*¹¹ measured the levels of total mercury and methyl mercury were measured in 40 tuna cans prepared from Persian Gulf tuna, in Tehran, Iran. The total mercury and methyl mercury concentrations were determined with a GBC 906 AA flame atomic absorption spectrometer (FAAS) and a Thermo gas chromatograph-mass spectrometer (GC-MS), respectively. The results showed that the means of total mercury and methyl mercury content of white and light style tuna were 280.48, 142.75, 229.05 and 113.37ppb, respectively.

Benjamin D. Barst *et al.*¹ determined mercury speciation in fish tissue with a direct mercury analyser. The method was validated by analysis of a certified reference material (DOLT-4 dogfish liver) and naturally contaminated fish tissues with comparison to an established Hg speciation method (gas chromatography cold vapor atomic fluorescence spectrometry). Recovery of organic Hg from DOLT-4, estimated by difference, averaged $99 \pm 5\%$ of the mean certified value for methylmercury. In most liver samples and all muscle samples, estimates of organic Hg from the proposed method were indiscernible from direct speciation measurements of methylmercury ($99\% \pm 6\%$). Estimation of organic Hg by the difference between total Hg and inorganic Hg was less accurate in liver samples with a high percentage of inorganic Hg (90%). This was because of the increased uncertainty that results from estimating a third value (i.e., organic Hg) by using the difference between two large concentrations (inorganic and total Hg). The proposed method is a useful tool for examining the speciation of Hg in fish muscle and liver, and by extension, potentially other tissues and environmental media.

Brian K. Niece and James F. Hauri² proposed a method of adapting an existing flame atomic absorbance spectrometer for this technique with little additional cost, thus allowing students to learn about this important technique. Students measured mercury concentrations in swordfish and tuna purchased at a local supermarket.

M. Stancheva *et al.*¹² determined heavy metals (Pb, Cd, As and Hg) in Black Sea grey mullet (*Mugil cephalus*). The fish samples were collected from two different Black sea areas – Varna Lake and Nesebar. The sample preparation was performed by acid microwave digestion with, Multiwave “system in five stages program. Determination of As, Cd, and Pb were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer, whereas Hg was analyzed by Milestone Direct Mercury Analyzer. Detected levels of As in the studied regions gives exceed those of other analyzed elements. The samples from both regions showed the higher levels of As in edible tissue than gills, especially from Region of Nesebar (1.1 mg/kg w.w.). The results for other heavy metals are several times lower than arsenic and were found in range 0.01–0.12 mg/kg w.w. All studied elements (except As) presented higher amounts from Varna Lake grey mullet compared with Nesebar region samples.

Paula M. Moraes *et al.*⁹ developed a simple, rapid and sensitive method for the determination of mercury concentrations in the muscle tissue of fish from the Brazilian Amazon using graphite furnace atomic absorption spectrometry (GFAAS) following acid mineralization of the samples in an ultrasonic cold water bath. Using copper nitrate as a chemical modifier in solution and sodium tungstate as permanent modifier, we were able to attain thermal stabilization of the mercury up to the atomisation temperature of 1600 °C in the GFAAS assay. The calculated limits of detection (LOD) and quantification (LOQ) were 0.014 and 0.047 mg kg⁻¹, respectively.

The main purpose of our research is to investigate optimal condition for determination of mercury in seafood products by applying the atomic emission spectrophotometry so that a reliable, efficient, sensitive and accurate protocol can be approached.

MATERIAL AND METHOD

Material & equipment

Shrimp and Pangasius products are collected in Mekong River Delta. The atomic emission spectrophotometry is utilized from Shimadzu, Japan.

Research method

- Optimize conditions for determine mercury in AES
- Effect of some reduced agents to the determination
- Effect of reduced medium to the determination
- Linear range of the regression equation
- Application for mercury determination in real seafood products

Statistical analysis

All data are processed by Excell.

RESULT AND DISCUSSION

Effect of the operating parameter in AES

The peak current is measured at 193.7nm which is specific for mercury. So we choose thi signal for investigation. The speed current appears in the cathode lamp at the current speed 60-85% to maintain the reliability and repeatably of the measurement.

Table 1. Effect of the speed current HCl to the emission degree

I, mA	6	7	8
Emission degreee	0.1307	0.1278	0.1252
RSD (%)	6.08	2.47	2.02

From the above table, we decide to choose the speed current 7mA to get the best signal.

Height of the atomic emission

To investigate the effect of height of the atomic emission to the emission degree, we examine the height 12 – 20mm. Our observation shows that 16 mm is appropriate for further experiment. Among different gases experimented, we see C₂H₂ at 1.8L per minute has the best emission signal.

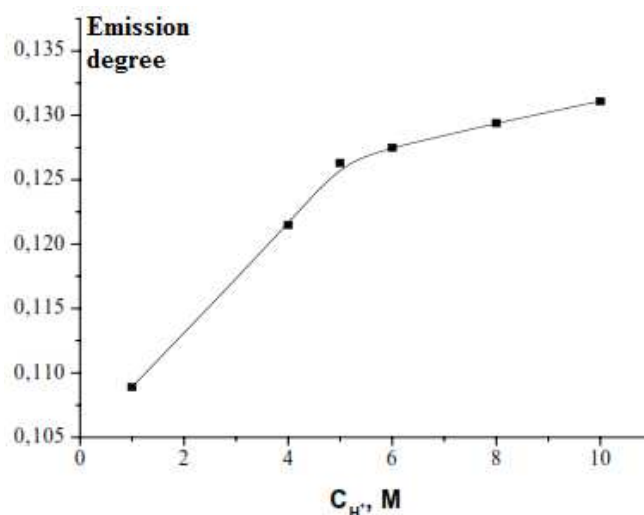
Effect of the reduced agent and medium

Concentration and kind of acid medium

Table 2. Effect of H⁺ concentration to emission degree of mercury

C_{H⁺}, M	1	4	5	6	8	10
Emission degree	0.1089	0.1225	0.1263	0.1275	0.1294	0.1311
RSD (%)	4.12	2.57	3.40	2.15	2.24	3.51

Fig. 1: Effect of H⁺ concentration of acid medium to emission degree of mercury



At the H⁺ 6M, we see the best peak current so we choose this value for further experiments.

Table 3. Effect of acid medium to emission degree of mercury

Solution	HCl 6M	H₂SO₄ 3M	HNO₃ 6M	H₃PO₄ 6M
Emission degree	0.1281	0.1257	0.0713	0.1071
RSD (%)	1.14	2.37	4.05	1.22
% Emission	100	98	56	84

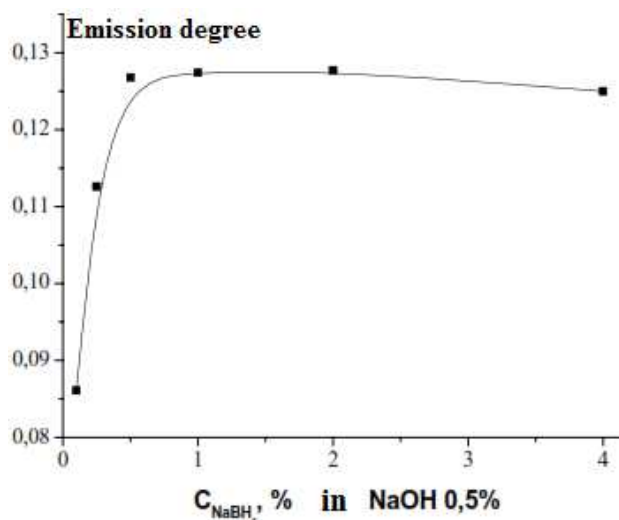
So we choose HCl 6M as the reduced medium during the mercury analysis.

Effect of concentration and injection speed of NaBH₄ to analysis

Table 4. Effect of the NaBH₄ concentration to the emission degree

NaBH ₄ , %	0.1	0.25	0.5	1	2	4
Emission degree	0.0861	0.1126	0.1268	0.1274	0.1277	0.1250
RSD (%)	4.2	5.5	2.8	3.5	4.9	7.2

Fig. 2: Effect of the NaBH₄ concentration to the emission degree

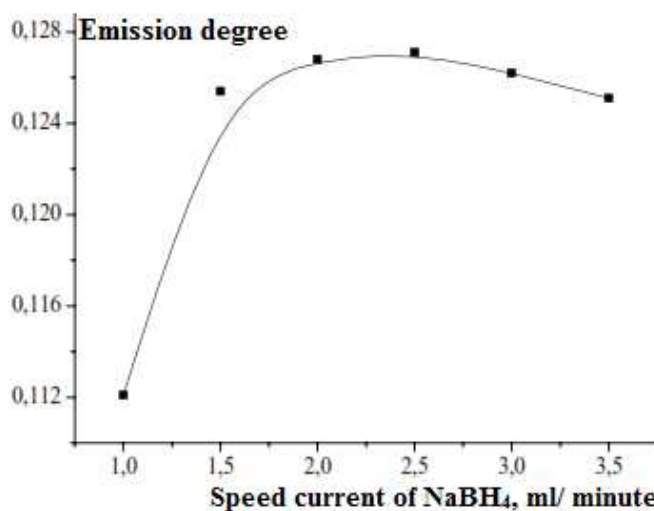


Effect of the speed current NaBH₄ to the reduced emission current

Table 5. Effect of the speed current NaBH₄ to the reduced emission current

Speed current, ml/minutes	1	1.5	2	2.5	3	3.5
Emission degree	0.1121	0.1254	0.1268	0.1271	0.1262	0.1241
RSD (%)	1.5	4.4	3.9	2.2	5.7	3.1

Fig. 3: Effect of the speed current NaBH₄ to the reduced emission current



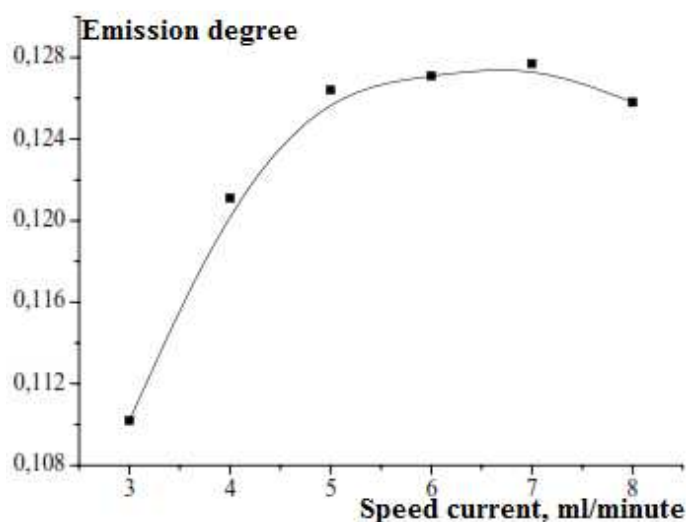
We see the speed current NaBH₄ 2 – 2.5ml/minute show the stable signal so we choose this value for further experiments.

Effect of the speed current to the emission measurement

Table 6. Effect of the speed current to the emission measurement

Speed current, ml/minutes	3	4	5	6	7	8
Emission degree	0.1102	0.1211	0.1264	0.1271	0.1277	0.1258
RSD (%)	4.7	5.1	1.2	4.6	4.2	6.5

Fig. 4: Effect of the speed current to the emission measurement



From above result, we see the speed current 7 ml/ minutes having the best measurement with high sensitivity and reliability.

Linear range and the calibration curve

Table 7. Emission degree of the measurement

Mercury, ppb	0	0.2	0.5	1	2	3	4
Emission	0	0.0072	0.0171	0.0334	0.0655	0.0952	0.1281
Mercury, ppb	5	7	8	10	12	15	18
Emission	0.162	0.2341	0.2571	0.315	0.3542	0.3841	0.4102

Fig. 5: Emission degree of the measurement

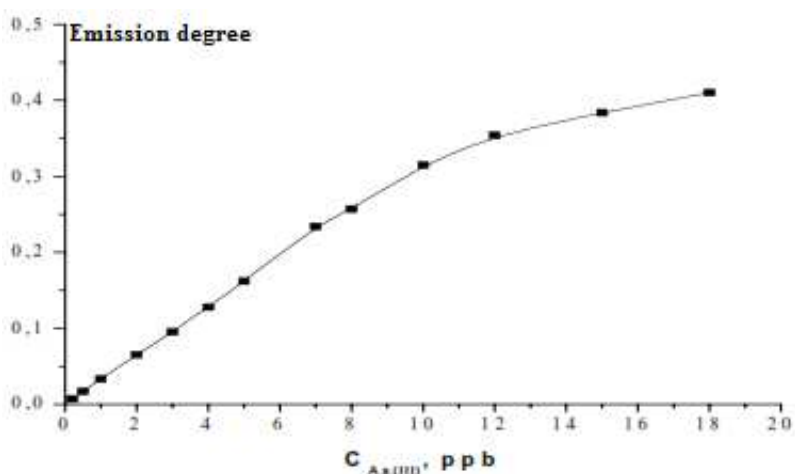
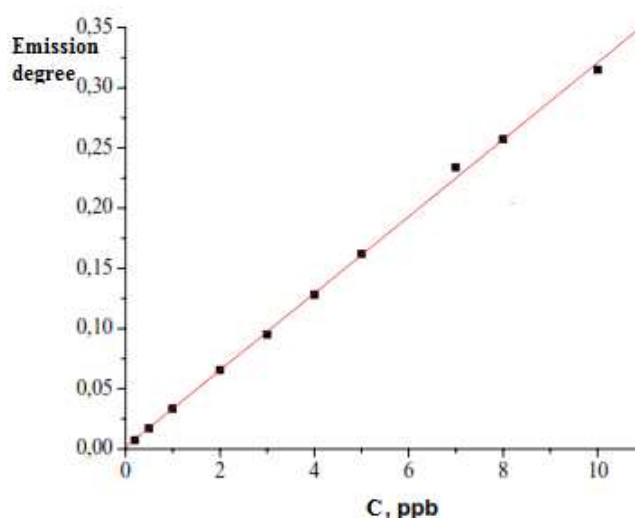


Fig. 6: Calibration curve of the measurement



Limit of detection LOD = 0.04(ppb); limit of quantification: LOQ = 0.15(ppb)

Application for mercury determination in real seafood products

We conduct 20 shrimp and 20 *Pangasius* samples collected in Mekong River Delta to survey the residual situation of mercury. Our results show that the residue of this heavy metal is in acceptable limit.

CONCLUSION

Mercury (Hg) is one of the most important pollutants both because of its effect on marine organisms and because it is potentially hazardous for humans. The toxicology and environmental behavior of Hg are complex since its toxicity, mobility, and bioaccumulation depend on its chemical form. So it's very necessary to find out a reliable scientific testing to determine this heavy metal in seafood product. This research can be considered for real application in seafood processing factory.

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