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Methylmercury and inorganic mercury determination in fish by cold vapour generation atomic absorption spectrometry

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Abstract

Given that organic mercury is more dangerous than the inorganic form and that it is converted into methylmercury by biological methylation, we have studied and optimized a simple method for measuring both organic and inorganic mercury contents in fish, using a spectroscopic vapour generation technique, with a sequential reduction of the digested sample with stannous chloride and sodium tetrahydroborate. Prior to applying the method the sample was subjected to alkaline wet digestion. Due to the matrix interferences calibration curves with matrix addition were needed for mercury determinations. The analytical parameters of the method were: linearity from 10 to 200 ng of Hg in the reduction vessel; detection limit: 125 and 183 ng/g fresh sample for inorganic mercury and methylmercury, respectively; precision (RSD%): 9.8 and 10.1 for inorganic mercury and methylmercury, respectively; accuracy: reference material (Dorm-2-NRC-CNRC) for methylmercury; value found 4504±272 ng/g; certified value 4470±320 ng/g. The method offers the advantage of not requiring special equipment to measure inorganic and organic mercury simultaneously in a sample. To evaluate its usefulness it was applied to nine different types of fish and mussels.

Keywords: Inorganic mercury; Methylmercury; Fish; Cold vapour AAS

1. Introduction

Mercury toxicity is highly dependent on its chemical form. Inorganic mercury is converted to methylmercury by biological methylation. This organic form is more dangerous than the inorganic one. In adult humans organic mercury damages specific cell types from areas such as the visual cortex and the cerebellum (Clarkson, 1998). Long-term, frequent intake of seafood with high mercury levels by populations living in coastal fishing villages is associated with a toxic risk, especially in pregnant women, because prenatal exposure disrupts the normal developmental processes of the fetal brain (Clarkson; Renzoni, Zino & Franchi, 1998).

Given the different toxicities of inorganic and organic mercury compounds, it is necessary to differentiate the two. The analytical techniques most widely used to do this are: gas–liquid chromatography with spectrometric detection, such as atomic fluorescence spectrometry (CG–AFS) (Cai, Monsalud, Furton, Jaffe & Jones, 1998), atomic emission spectrometry (CG–AES) (Enteborg, Sinemus, Radzuik, Baxter & French, 1996; Gerbersmann, Heisterkamp, Adams & Broekaert, 1997; Pereiro, Wasik & Lobinski, 1998), and voltammetric techniques (Lai, Huang, Zhou & Wipf, 1998). Usually the methods applied to mercury speciation require two different sample treatments in order to determine, first, the total mercury and then extract or separate the organic mercury for its determination.

The purpose of our work is to propose a simplified, and optimized method for measuring organic and inorganic mercury contents in fish that takes the spectrophotometric method proposed by Oda and Ingle

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(1981) as a starting point. The method uses a vapour generation technique, with a sequential reduction, in the same reaction vessel, of the inorganic and organic mercury, using $SnC1_2$ and $NaBH_4$, respectively.

2. Methods and materials

2.1. Instrumentation

The following apparatuses were used: a Perkin-Elmer 2380 atomic absorption spectrophotometer, equipped with a quartz absorption cell (19 mm long, 17 mm i.d. with quartz windows) and a hollow cathode mercury lamp; a hydride generation vessel (50 ml capacity); a Heracus 1P20 forced air oven and a small fishtank air pump.

2.2. Reagents

All reagents were of analytical grade. Hydrochloric acid 37% (sp.gr. Merck); nitric acid 65% (sp.gr. 1.40 Merck); potassium dichromate 99.5% (Panreac); potassium hydroxide (Merck); sodium chloride 99.5% (Panreac); sodium tetrahydroborate 98–99% (Sigma); sodium thiosulphate 97% (Merck); stannous chloride 99% (Panreac); antifoaming silicone (Merck).

Inorganic mercury stock solution (Titrisol Merck) 1000 mg/l; Methylmercury 95% (Alfa): Methylmercury stock solution 1000 mg/l was prepared weighing 0.1317 g of methylmercury in ethyl alcohol to 100 ml. Standard working solutions (100 ng/ml) were prepared from an intermediate solution of 10 mg/l.

Oxidizing solution: 1 g of potassium dichromate with 20 ml of nitric acid and deionized water to l00ml.

Deionized water was used (Milli Q System, Millipore). All glassware was soaked in nitric acid for 15 minutes and rinsed with deionized water before use.

2.3. Samples

To optimize the method, fish samples and standard reference material DORM-2 (dogfish-muscle) from the National Research Council Canada/Conseil National de Recherches Canada (NRC-CNRC) were used. The method was applied to nine different fish species and mussels from the Valencian Community market. All samples were dried in an oven at 60°C for 24 h and then ground in a glass mortar.

2.4. Procedure

0.25–0.3 g of the dried sample were weighed in an assay tube fitted with a screw stopper. Two and a half milliliters of KOH 10 M were added, and the mix was introduced into a boiling water bath for 25 min. After digestion the sample was allowed to cool and transferred into a l00 ml volumetric flask; the tube was washed with 1% (w/v) NaC1 solution, and then 7.5 ml of nitric acid and 0.5 ml of oxidizing solution were added and the volume was completed to 100 ml with 1% (w/v) NaCl solution. The obtained

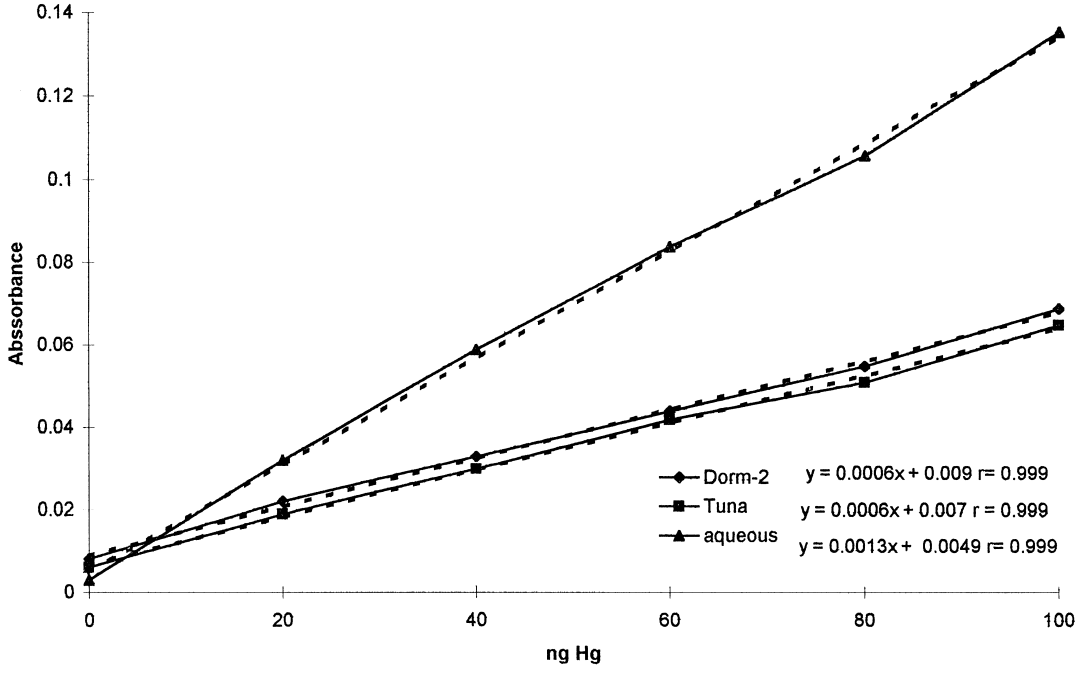

Fig. 1. Inorganic mercury: assay results for matrix interference.

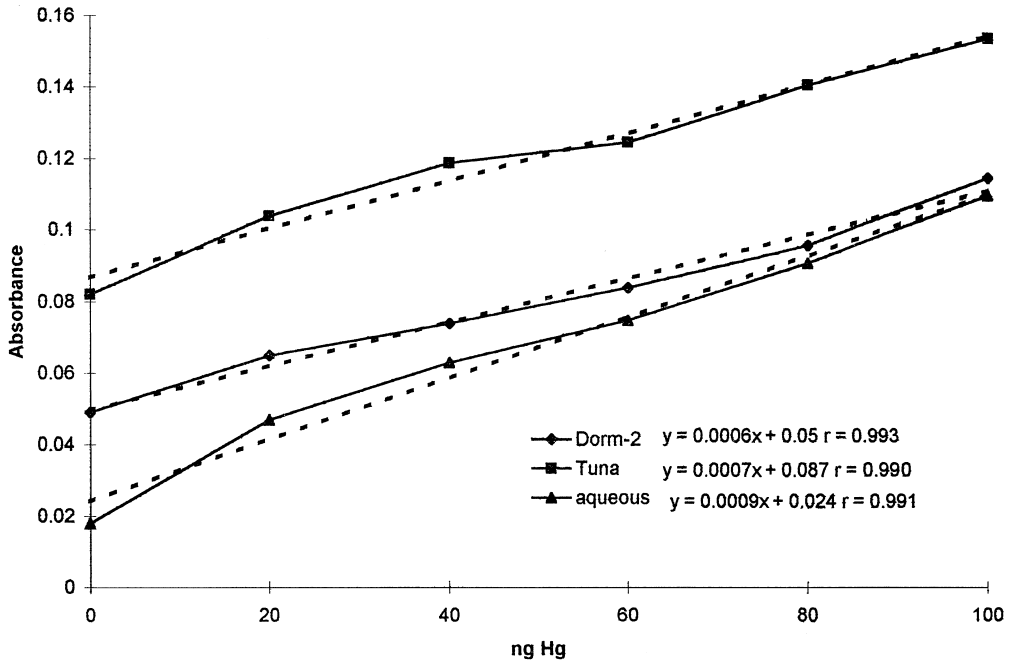

Fig. 2. Organic mercury: assay results for matrix interference.

solution was used for inorganic and organic mercury determination.

Mercury was measured by cold vapour atomic absorption spectrophotometry ($\lambda = 253.5$ nm lamp intensity 7 mA slit width 0.7 nm). A silicone drop was added to 5 ml of the digested solution which was transferred to the reduction vessel (a wash bottle of 50 ml of capacity), 1 ml of 1% (w/v) $SnCl_2$ solution was added, and then air was vigorously bubbled into the mixture. The measured peak height corresponded to the inorganic mercury, while the organic mercury remained in the vessel. Once the signal had gone to the base line, 0.5 ml of the oxidizing solution followed by 1 ml of 1% (w/v) $NaBH_4$ were added. The signal corresponded to the organic mercury present in the vessel.

In order to check the analytical quality of the method the analytical parameters were determined and then the method was applied to the determination of organic and inorganic mercury contents in samples of fish bought in markets in the Valencian Community.

Table 1
Inorganic and organic mercury: detection limit

($n = 9$)[a]	In assay (ng)	Dry sample (ng/g)	Fresh sample[b] (ng/g)
Inorganic Hg	6.25	417	125
Methyl-Hg	9.13	608	183

[a] n = Number of blanks assayed.
[b] Mean moisture content 70%.

Table 2
Accuracy: standard reference material

	Inorganic Hg	Methyl-Hg	Total Hg
Certified value (ng/g)	–	4470±320	4640±260
Found value (ng/g) ($n = 6$[a])	< D.L.[b]	4504±272	–

[a] n = number of samples.
[b] D.L. = detection limit.

3. Results and discussion

The equipment used for flow hydride generation is a modification of the one proposed by Oda and Ingle (1981). It is a flow system made up of a wash bottle, connected at one end to an air pump and at the other end to a quartz cell. This cell has an outlet that permits all vapours to be collected on a 1% sodium tiosulphate solution, in order to sweep, once measured, all the mercury generated.

Air-flow has an important effect on the measurements. We observed that the decrease of the flow improve the measure; therefore a relatively low flow is recommended. With the pump used we obtained a flow of 1200 ml/min.

3.1. Analytical parameters

3.1.1. Linearity

The linearity of the response in the range from 10 to 200 ng of Hg in the reduction vessel was checked both with inorganic and organic mercury. Inorganic mercury $y = 0.00685 + 0.00107x$; $r = 0.997$ Methyl mercury $y = 0.0034 + 0.00098x$; $r = 0.995$. The usual working range was from 20 to 100 ng in the reduction vessel.

Table 3
Mercury contents of different samples of fish and mussels from Valencia markets ($n = 3$)

Samples	Origin	Product	Moisture (%)	Inorganic Hg (ng/g)		Organic Hg (ng/g)	
				Dry matter	Fresh product	Dry matter	Fresh product
Tuna[b]	Unknown	Fresh	70.63	(328) < D.L.[c]	96	7954±312	2336±92
Cod[a]	Unknown	Fresh	77.69	< DL.		(278) < D.L.	62±25
Kind of anchovy[a]	Valencia (Spain)	Fresh	77.52	< DL.		753±199	169±45
Swordfish[b]	Chile	Frozen	73.88	< D.L.		6053±65	1581±17
Sole[a]	Netherlands	Fresh	79.21	< D.L.		< D.L.	
Hake[a]	Unknown	Fresh	79.81	< D.L.		1190±137	240±28
Hake[a]	North (Spain)	Fresh	80.63	< D.L.		794±199	154±39
Hake[a]	Argentine	Frozen	78.33	< DL.		< D.L.	
Hake[a]	Namibia	Frozen	81.51	< DL.		(480) < D.L.	89
Hake[a]	Namibia	Frozen	82.38	< DL.		(377) < D.L.	66
Mussel[a]	Unknown	Fresh	75.89	< D.L.		< D.L.	
Mussel[a]	Tarragona (Spain)	Fresh	77.36	< D.L.		< D.L.	
Small hake[a]	Unknown	Fresh	83.59	< D.L.		(461) < D.L.	76±21
Sardine[a]	Unknown	Fresh	65.89	< DL.		< D.L.	
Salmon[a]	Unknown	fresh	67.56	< DL.		538±146	175±48

[a] Maximum allowed 0.5 mg/kg fresh product.
[b] Maximum allowed 1 mg/kg fresh product.
[c] D.L. = detection limit.

3.1.2. Matrix interferences

Application of the addition's method revealed matrix interferences. See Figs. 1 and 2, which correspond to the calibration curves with and without matrix addition of inorganic mercury (Dorm-2 or tuna) and organic mercury (Dorm-2 or tuna), respectively.

Due to the matrix interferences calibration curves with matrix addition were needed for mercury determinations.

3.1.3. Detection limit (AMC, 1987)

The detection limits (DL), defined as the mercury concentration corresponding to three times the standard deviation of nine reagent blanks are shown in Table 1 (5 ml of solution were introduced into the reduction vessel). The values obtained indicate that it is possible to measure mercury contents lower than the maximum allowed by the EEC (0.5 and 1 mg/kg of the edible portion of fresh product, depending of the fish considered) (EEC, 1993).

3.1.4. Precision

The precision of the method was estimated from the values obtained in the independent analysis of 10 aliquots (0.25 g) of a swordfish sample. Samples were digested, the volume completed to 100 ml and then 5 ml of this solution were introduced into the digestion vessel.

The values, expressed as percentage of the relative standard deviation, were: 9.8 for inorganic mercury (mean content = 406 ng/g dry weight, value lower than the DL) and 10.1 for methylmercury (mean content = 4409 ng/g dry weight). These values can be considered acceptable for a trace element measurement.

3.1.5. Accuracy

In order to check the accuracy of the studied method, the inorganic and organic mercury contents of a reference material DORM-2-NRC-CNRC were determined. The values obtained together with the certified values are reported in Table 2.

In the recovery assays, carried out on eight swordfish aliquots, 2667 ng/g of both inorganic and organic mercury was added to four of them. The recovery percentages were 90±14 and 108.9±12.7%, respectively (present mercury:—inorganic, not detectable;—organic, 4635 ng/g).

3.2. Mercury contents in fish marketed in the Valencian community

To evaluate the usefulness of the studied method in determining the mercury (organic and inorganic) contents at the levels usually found in fish products marketed in the Valencian Community, nine types of seafood (fish and mussels)(15 samples in total) bought at different selling points in the city of Valencia were analysed. The results are the mean values±standard deviations of the analysis of three portions of the same sample and are reported in Table 3.

4. Conclusions

The analytical parameters together with the values obtained in the analysed samples show the usefulness of the method for measuring organic mercury in fish products. It must be pointed out that the majority of the

analysed products had inorganic mercury contents lower than the detection limit of the method. The method offers the advantage of not requiring special equipment to measure inorganic and organic mercury simultaneously in a sample.

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