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Determination of mercury in dry-fish samples by microwave digestion and flow injection analysis system cold vapor atomic absorption spectrometry

L. Aduna de Paz, A. Alegría, R. Barberá, R. Farré & M. J. Lagarda

Nutrition and Food Chemistry, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

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Flow injection analysis system cold vapor atomic absorption spectrometry (FIAS-CV-AAS) preceded by a wet digestion in a microwave oven, as a method for measuring mercury in fish was studied. The digestion process and conditions of the FIAS (carrier concentration: HCl 3% v/v; reducing agent: $SnCl_2$ 2% w/v: filling and injection times: 8 and 25 min, respectively; and sample volumes) were optimized.

The analytical parameters of the proposed method (detection limit = 7.7 ng/g; precision intraassay = 6.7%; interassay = 14.0%) demonstrates its adequacy and are similar to the ones (detection limit = 19.4 ng/g; precision intraassay = 11.2%; interassay = 15.9%) obtained using a conventional wet digestion method with $HNO_3 + H_2SO_4 + V_2O_5$. The accuracy are verified with reference material DORM-1 (dog fish-muscle) NAC-CNRC. Moreover, with the optimized method the risk of losses and contamination is low and it is less time consuming and requires smaller reagent volumes than conventional method. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Mercury is a trace element that in small quantities can cause severe toxic effects. For people not exposed to mercury in their work, the most probable source of this element is their diet. The reported mercury content of food is rather low, about 0.02 μg/g, but there is a high variability in the content depending on the kind of product, its geographic origin and the agricultural and industrial techniques of the area taken into account. (Hugunin & Bradley, 1975)

Mercury is accumulated through the food chain, especially in an aquatic medium, where concentration factors of a hundred and even a thousand have been reported. Therefore, fish can have a higher mercury content than other foods, but it is difficult to give an average content, because that depends on the fish species considered, its age, size and the conditions of the water in which it lives (Hugunin & Bradley, 1975; De Sousa & López Goyanes, 1992). On the other hand, the mercury content of fish can increase in the preparation processes as a result of deshydratation when it is cooked, dried, pickled, salted. (De Sousa & López Goyanes, 1992).

A large number of studies on mercury determination have been published. The four most common and reliable techniques for mercury determination are spectrophotometry after chelation with dithizone; atomic absorption and emission spectroscopy; neutron activation analysis and gas chromatography. Of all these, cold vapor atomic absorption (CV-AAS) has probably become the most popular technique because the mercury compounds can be reduced to elemental mercury that occurs as a vapor (Lam Leung *et al.*, 1991; Landi *et al.*, 1992; Navarro *et al.*, 1992; Guo & Baasner, 1993; Adeloju *et al.*, 1994). The FIAS have proved to be an efficient alternative to the conventional mercury hydride system, because it gives improved sensitivity and selectivity (Vermeir *et al.*, 1991; Guo & Baasner, 1993).

Regardless of the digestion method used, the organic matter has to be destroyed, and if the CV-AAS technique is applied, all the mercury forms present must be oxidized to mercury (II) prior to reduction to elemental mercury. The wet digestion methods reported (Marts & Blaha, 1983; De Sousa & López Goyanes, 1992; Landi *et al.*, 1992; Guo & Baasner, 1993; Adeloju *et al.*, 1994) use strong acid (HCl, H_2SO_4, HNO_3) and oxidants (H_2O_2, $KMnO_4$, $K_2Cr_2O_7$).

The disadvantages of the wet digestion methods are well known: possible losses of mercury due to the high temperatures; the large amounts of reagents that are needed, responsible for high blank values that impair the detection limits. Wet ashing in a closed vessel in a microwave oven has been found to be an efficient method for preparing samples, because the losses of mercury and the time needed for the determination are reduced (Farré *et al.*, 1991; Lam Leung *et al.*, 1991; Navarro *et al.*, 1992).

The aim of this work is to study a flow injection analysis system cold vapour atomic absorption spectroscopic method which includes a microwave wet digestion (using teflon vessels) for determining mercury in fish. The conditions of wet digestion and FIAS (carrier, reducing agent, volume of sample etc.) were optimized and the analytical parameters of the described method were compared with a conventional method similar to the one proposed by Marts & Blaha (1983).

EXPERIMENTAL

Instrumentation

A Perkin–Elmer model 2380 atomic absorption spectrophotometer in conjunction with a Perkin–Elmer FIAS-100 was used. The system was operated through personal computer and the associates softwear. Conventional hollow cathode lamp was used for mercury. A hot block digestor: a Kjeldatherm Gerhardt block digestion system. Microwave digestion system Milestone MLS 1200 with medium pressure vessel MV 100 with burst disc.

Reagents

All reagents were of analytical reagent grade. Hydrochloric acid 37% (sp.gr.1.33 Probus); nitric acid 65% (sp.gr.1.40 Merck); sulfuric acid 96–97% (sp.gr.1.84 Merck); sodium tetrahydroborate 98–99% (Sigma); stannous chloride 99% (Panreac); vanadium pentoxide (Sigma); hydrogen peroxide 33% (Panreac).

Mercury stock solution (Titrisol Merck) 1000 mg/l ($Hg(NO_3).H_2O$ in HNO_3 0.5M). Standard working solutions were prepared from stock solution inmediately before use. Water was glass distilled and deionized (Milli-Q System, Millipore). All glassware was soaked in nitric acid for 10 mins and rinsed with deionized water before use.

Samples

One Kg each of dried and salted codfish fillets, fresh hake and salmon was bought in a local supermarket. The fresh and dry samples were chopped up fine by hand and homogenized and the fresh ones were dried in a oven (100°C). Standard reference material DORM-1 (dogfish-muscle) NAC-NRCC.

Procedure

Microwave digestion of samples

In order to choose the optimum conditions for the digestion, different amounts of sample, ratio of nitric acid and hydrogen peroxide, times and power were assayed. The best results were obtained by placing an amount in the range of 0.1–0.4 g of sample in the medium pressure Teflon vessels of the microwave digestion system and then adding 4 ml of nitric acid and 0.2 ml of hydrogen peroxide. The two step digestion program was as follows

1. first step: 4 mins at 300 W (25%);
2. second step: 2 mins at 600 W (50%).

The volume was completed, after digestion to 5 ml with water.

Conventional digestion of samples

4 ml of nitric acid and 40 mg of vanadium pentoxide were added to a 2 g sample placed in the digestion tube. Samples were heated at 140°C in the block for 5 mins. The temperature must be controlled to prevent losses of mercury. After cooling, 7.5 ml of sulfuric acid was added and the tubes were heated at 140°C for 15 mins. Then, 5 ml sulfuric acid 2 M was added and heated again for 5 mins. After cooling, the digestion products were transferred to 25 ml volumetric flasks and were diluted to volume with deionized water.

RESULTS AND DISCUSSION

Optimization flow injection analysis system (FIAS-CVAAS)

1. Different reducing agent, concentration of reducing agent and carrier and volume of sample were assayed to choose the best conditions. The reducing agent studied were stannous chloride 0.7, 1.1 and 1.5% (v/v) dissolved in 3%(v/v) hydrochloric acid and sodium tetrahydroborate 0.04, 0.05 and 0.1%(w/v) in sodium hydroxide 0.1%(w/v). The carrier assayed was hydrochloric acid 2, 3 and 4% (v/v).Two sample volume were assayed 500 and 200 μl, and the highest absorbance value was obtained for a sample volume of 500 μl, which requires a minimum filling time of 8 s. The values obtained for a standard of 10 ng/ml of mercury are shown in Figs 1 and 2.
2. The most sensitive signals were obtained with HCl 3% (v/v) and stannous chloride 1.1% (w/v) and sodium tetrahydroborate 0.05% (w/v). The analysis of variance was applied to the results obtained with stannous chloride 1.1% and sodium tetrahydroborate 0.05%. Since no significant difference were found ($\alpha = 0.05$), stannous (II) chloride was chosen for it is more stable and easier to prepare than the sodium tetrahydroborate.

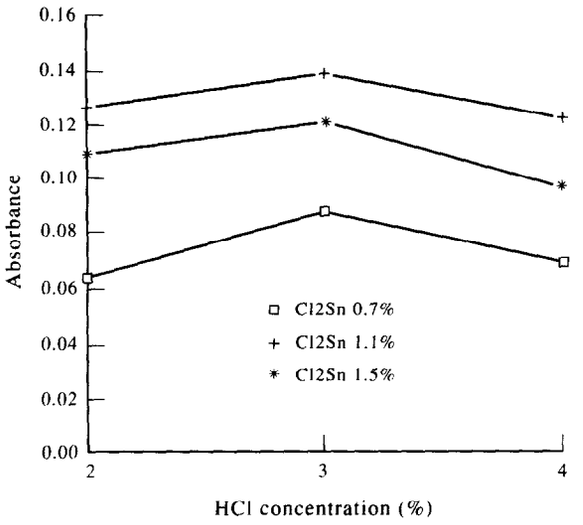

Fig. 1. Optimization of hydrochloric acid and stannous chloride concentrations.

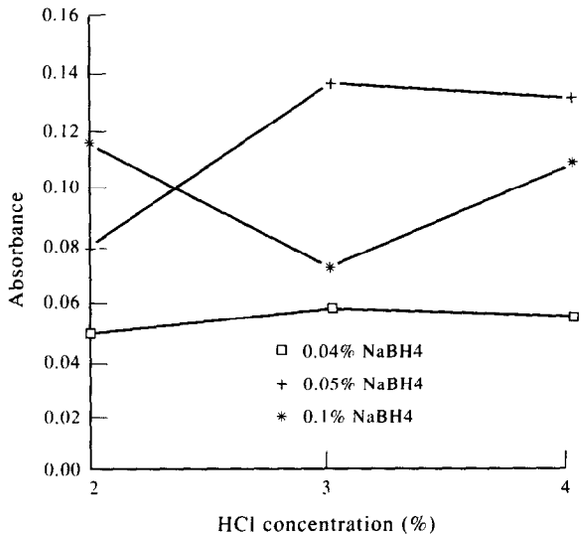

Fig. 2. Optimization of hydrochloric acid and sodium tetrahydroborate concentrations.

When the conditions optimized for an aqueous standard of mercury were applied to the digested samples, the measurements varied greatly, regardless of the digestion method applied. Therefore, an optimization of the concentration of reducing agent in samples and standards subjected to the same digestion process was carried out. Absorbance values of cod sample minus blank reagent values plotted against reducing agent concentrations are reported in Fig. 3. The absorbance values and, therefore, the mercury contents increase when the stannous chloride concentration increases from 1.1 to 2% and then the values reach a plateau. The optimum conditions are shown/ reported in Table 1.

In order to check the applicability of the method to other fish species, it was used to measure the mercury content of hake and salmon. Samples were previously

Table 1. Instrumental conditions by mercury determination

Atomic absorption spectometer conditions[a]	
Wavelength (nm)	253.7
Slit width (nm)	0.7
Intensity lamp (mA)	6
Flow-injection automatic conditions[b]	
Carrier	HCl 3%(v/v)
Flow-rate of carrier	9 ml/min
Reducing agent	$SnCl_2$ 2%(w/v) in HCl 3%(v/v)
Flow-rate of reducting agent	5 ml/min
Volume of sample (μl)	500
Filling time (s)	8
Read time (s)	25

[a]Read peak-height.
[b]Carrier gas used was argon 100 ml/min.

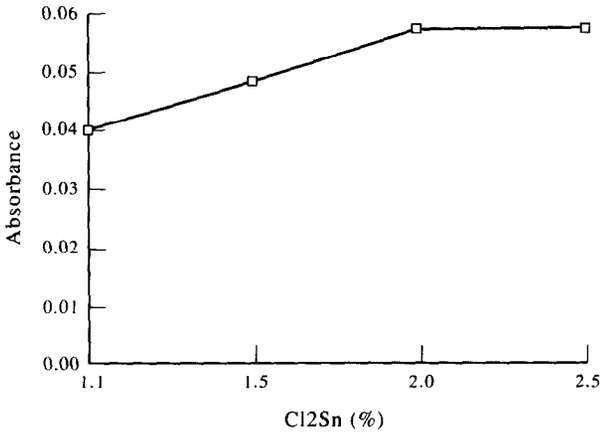

Fig. 3. Optimization of stannous chloride concentrations in sample.

dried. The mean and standard deviations for three determinations of the same dried sample were 1.13 ± 0.056 μg/g and 39.60 ± 3.54 ng/g for hake and salmon, respectively. Variation coefficients were similar to those obtained for cod fish.

Comparation of the microwave digestion method with the conventional wet digestion

To check the quality and usefulness of the microwave digestion method it was compared with the conventional one, by estimating the analytical parameters of both.

Linearity

The linearity of the response was verified using standads that ranged from 2 to 40 ng/ml. The adjusted lineal equation and correlation coefficient obtained was:

Microwave digestion method

$y = 0.0065 \times +0.005 \qquad r = 0.998;$

Conventional digestion method

$y = 0.0062 \times +0.022 \qquad r = 0.996.$

Detection limit (ACS, 1983)

Detection limit, defined as the mercury concentration corresponding to three times the standard deviation of eight reagent blanks were 7.7 ng/g for microwave digestion method and 19.4 ng/g for conventional digestion method. Both values are low enough to allow determination of the mercury content of the samples taken into account, always higher than 100 ng/g. However, the detection limit of microwave digestion method is lower than that of conventional digestion method.

Precision

The instrumental precision was estimated from 10 consecutive measures of the same dilution of a digested sample. The lowest values was obtained for microwave digestion method (1.1%) whereas by conventional digestion method the value was 2.6%, for a average mercury concentrations of 149 ng/g. The precision of the method was estimated from an analysis of eight homogenous aliquots of a cod fish sample. The intra-assay precision (aliquots of a sample digested and measured during the same assay session) and inter-assay precision (aliquots of the same sample digested and measured in different days) were also estimated. The values obtained for a mean mercury content of 140 ng/g were:

Microwave digestion method; Intraassay 6.67% and interassay 14.01%,

Conventional digestion method; Intraassay 11.2% and interassay 15.9%.

Accuracy

In order to verify the accuracy of the assayed methods, the mercury content of a reference material DORM-1 (dogfish-muscle) NAC-CNRC (certified value 798 ± 74 ng/g) was determined. The results μg/g found were:

Microwave digestion method; 782 ± 73.8;

Conventional digestion method; 751 ± 98.

CONCLUSIONS

From the values of the analytical parameters of the proposed method (microwave digestion and flow-injection cold vapor atomic absorption spectrometry) it can be concluded that it is useful for determining mercury in fish and gives a lower detection limit than the conventional method. The main advantages of microwave digestion when compared with the conventional organic matter destruction are: a four times shorter digestion time, a lower risk of losses by volatilization and the use of smaller reagent volumes that reduces the risk of contamination.

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