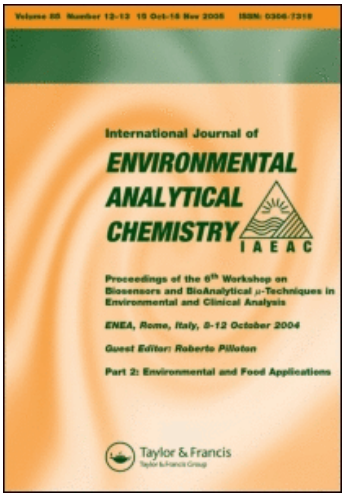

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Total and inorganic mercury determination in fish tissue by flow injection cold vapour atomic fluorescence spectrometry

Gabriel G. A. de Carvalho[ab]; Mário A. Feres Jr[a]; José R. Ferreira[ac]; Valerie H. Kennedy[d]
[a] Analytical Chemistry Laboratory, Nuclear Energy Center for Agriculture, São Paulo University, Piracicaba, Brazil [b] Health Science Faculty, Methodist University of Piracicaba, Piracicaba, Brazil [c] The Regional South Research Center-APTA, Agriculture Secretary, Piracicaba, São Paulo [d] Centre for Ecology & Hydrology - Lancaster, Lancaster Environment Centre, Lancaster, United Kingdom

Online publication date: 11 June 2010

To cite this Article de Carvalho, Gabriel G. A. , Feres Jr, Mário A. , Ferreira, José R. and Kennedy, Valerie H.(2010) 'Total and inorganic mercury determination in fish tissue by flow injection cold vapour atomic fluorescence spectrometry', International Journal of Environmental Analytical Chemistry, 90: 9, 686 — 696
To link to this Article: DOI: 10.1080/03067310902871729
URL: http://dx.doi.org/10.1080/03067310902871729

Intern. J. Environ. Anal. Chem.
Vol. 90, No. 9, 10 August 2010, 686–696

Taylor & Francis
Taylor & Francis Group

Total and inorganic mercury determination in fish tissue by flow injection cold vapour atomic fluorescence spectrometry

Gabriel G.A. de Carvalho[ab]*, Mário A. Feres Jr.[a], José R. Ferreira[ac] and Valerie H. Kennedy[d]

[a]*Analytical Chemistry Laboratory, Nuclear Energy Center for Agriculture, São Paulo University, Piracicaba, Brazil;* [b]*Health Science Faculty, Methodist University of Piracicaba, Piracicaba, Brazil;* [c]*The Regional South Research Center-APTA, Agriculture Secretary, Piracicaba, São Paulo;* [d]*Centre for Ecology & Hydrology – Lancaster, Lancaster Environment Centre, Lancaster, United Kingdom*

(*Received 20 August 2008; final version received 25 February 2009*)

A simple and reliable method for Hg determination in fish samples has been developed. Lyophilised fish tissue samples were extracted in a 25% (w/v) tetramethylammonium hydroxide (TMAH) solution; the extracts were then analysed by FI-CVAFS. This method can be used to determine total and inorganic Hg, using the same FI manifold. For total Hg determination, a 0.1% (w/v) $KMnO_4$ solution was added to the FI manifold at the sample zone, followed by the addition of a 0.5% (w/v) $SnCl_2$ solution, whereas inorganic Hg was determined by adding a 0.1% (w/v) L-cysteine solution followed by a 1.0% (w/v) $SnCl_2$ solution to the FI system. The organic fraction was determined as the difference between total and inorganic Hg. Sample preparation, reagent consumption and parameters that can influence the FI-CVAFS performance were also evaluated. The limit of detection for this method is $3.7\,ng\,g^{-1}$ for total Hg and $4.3\,ng\,g^{-1}$ for inorganic Hg. The relative standard deviation for a $1.0\,\mu g\,L^{-1}$ CH_3Hg standard solution ($n=20$) was 1.1%, and 1.3% for a $1.0\,\mu g\,L^{-1}$ Hg^{2+} standard solution ($n=20$). Accuracy was assessed by the analysis of Certified Reference Material (dogfish: DORM-2, NRCC). Recoveries of 99.1% for total Hg and 93.9% inorganic Hg were obtained. Mercury losses were not observed when sample solutions were re-analysed after a seven day period of storage at 4°C.

Keywords: mercury speciation; total mercury; inorganic mercury; L-cysteine; fish; tetramethylammonium hydroxide; atomic fluorescence spectrometry; flow injection

1. Introduction

The toxicity of elements such as Hg is directly related to its chemical form. Methylmercury (CH_3Hg) is one of the most important Hg species in terms of risk to the biota [1–4] because it can be absorbed through biological membranes and accumulate in animal tissue. Organic Hg is incorporated into fish enzymes and proteins by binding to sulphydryl groups [3,5], mainly in the central nervous system, and can lead to neurotoxity [6]. These stable CH_3Hg compounds are eliminated from animal tissues more slowly

*Corresponding author. Email: ggcarvalho@cena.usp.br

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310902871729
http://www.informaworld.com

than inorganic Hg. Total Hg is therefore an unreliable indicator of Hg residence time in animal tissue [7,8]. Thus, analytical methods involving separation of CH_3Hg from inorganic Hg in biological samples are of utmost importance, as Hg toxicity is dependent upon concentration and chemical form of the element [8,9].

Methods for Hg speciation in environmental and biological samples are well documented. In general, they include liquid or gas chromatography and capillary electrophoresis, coupled to different techniques such as: atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma–mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES) and microwave-induced plasma optical emission spectrometry (MIP-OES) [10]. The cold vapour (CV) generation coupled to AFS is considered one of the most sensitive and low-cost analytical tools for this task [11]. Kaercher *et al.* [12] and Torres *et al.* [13] have developed an Hg speciation method based upon vapour generation, and selective detection of either inorganic, or total mercury controlled by temperature variation of the quartz AAS cell.

The use of L-cysteine for Hg speciation in environmental and biological samples has also been investigated. In general, the Hg species are attached by the thiolic group from L-cysteine, which can then be separated for further detection. L-cysteine is also used as an eluent in chromatography and other analytical methods [10,14–19].

Tao *et al.* [14,20] described a method for the determination of inorganic and total Hg in fish tissue which does not require chromatographic separation and uses flow injection (FI) CVAAS. For total Hg, the organomercury species are decomposed by an on-line addition of potassium permanganate [20–24], and the inorganic Hg is released from the sample solution by the addition of L-cysteine [14]. Park and Do [25] determined total Hg by using FI-CV ICP-MS without the on-line addition of oxidisers; the organic Hg species were reduced using $NaBH_4$.

The alkaline solubilisation of biological materials with tetramethylammonium hydroxide (TMAH) is well documented, leading to simple sample preparation procedure in trace element analysis [26–28], including Hg determination [13,14,20,24,25,29–33]. The use of TMAH solubilisation with an FI system, reduces both analyte losses and sample contamination.

The aim of this paper was to improve the on-line determination of total and inorganic Hg in fish tissues, using a simple sample preparation method with TMAH, at room temperature. The injected sample reacts either with $KMnO_4$ or with L-cysteine solutions, with a further addition of $SnCl_2$ reductant solutions in a FI manifold, prior to Hg quantification by CVAFS. The FI manifold was optimised for sensitivity, reagent consumption and simplicity.

2. Experimental

2.1 *Instrumental*

Total and inorganic Hg were measured by using an atomic fluorescence detector (10.023 Merlin, PS Analytical, Kent, UK) powered by a boosted mercury lamp, coupled to a photomultiplier tube with maximum sensitivity at 253.7 nm; a dryer tube (Nafion®) 20 cm long was connected to the outlet of the quartz gas-liquid separator (GLS) to minimise water vapour seepage into the detector. Signal responses were achieved in peak heights and were processed by a dedicated software package (Avalon®, PS Analytical, UK).

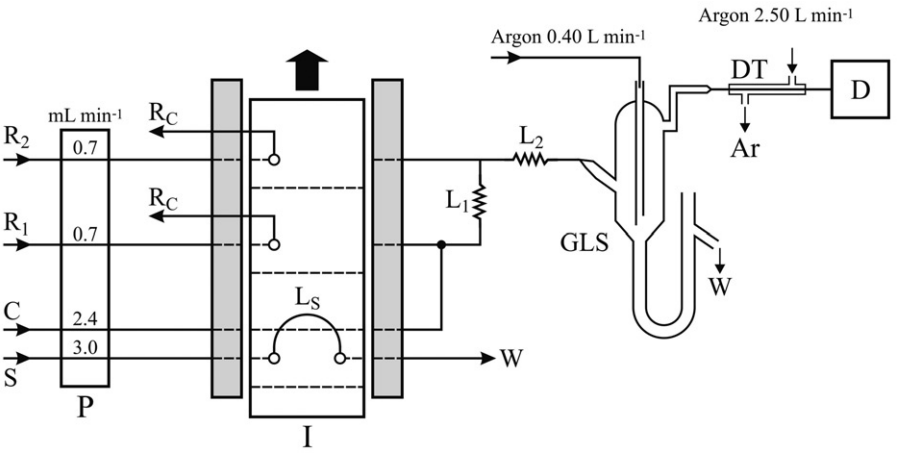

Figure 1. Diagram of the flow system used for the determination of total and inorganic Hg by FI-CVAFS in fish samples. S = sample; C = carrier stream (0.1 mol L^{-1} HCl); R_1 = 0.1% (w/v) $KMnO_4$ (total Hg) or 0.1% (w/v) L-cysteine (inorganic Hg); R_2 = 0.5% (w/v) $SnCl_2$ (total Hg) or 1.0% (w/v) $SnCl_2$ (inorganic Hg); R_C = recycling; P = peristaltic pump; I = injector; Ls = 1,000 μL; L_1 = 20 cm; L_2 = 30 cm; GLS = gas-liquid separator; DT = dryer tube (Nafion®); D = AFS; W = waste.

2.2 *The flow injection system*

The flow injection manifold (Figure 1) comprised one peristaltic pump (Ismatec® IPC), a homemade manual injector [34] and an atomic fluorescence detector. The system configuration was established according to Tao *et al.* [20] and was optimised for sensivity and simplicity. As recommended by Reis *et al.* [35] Teflon® tubes (0.8 mm internal diameter) and pumping tubes (Tygon®) of different diameters were used in the FI system. For sample dispersion optimisation, sample loops of 500, 750 and 1,000 μL, argon flow rates in the range of 0.20 to 0.60 L min^{-1} and sample carrier stream flow rates from 1.8 to 9.2 mL min^{-1} were used. Reagent concentrations were also investigated; these varied from 0.00 to 1.00% (w/v) for L-cysteine, 0.10 to 0.75% (w/v) for $KMnO_4$, and from 0.25 to 5.00% (w/v) for $SnCl_2$.

For total mercury, the sample was injected into the sample carrier stream (0.1 mol L^{-1} HCl) and merged sequentially with 0.1% w/v $KMnO_4$ and 0.5% (w/v) $SnCl_2$ solutions. Elemental Hg^0 vapour was separated in the GLS and transported towards the atomic fluorescence spectrometer by argon flow (Figure 1).

Inorganic Hg determination was carried out by using 0.1% w/v L-cysteine and 1.0% (w/v) $SnCl_2$ solutions instead of 0.1% (w/v) $KMnO_4$ and 0.5% (w/v) $SnCl_2$ solutions. Total and inorganic Hg analytical signals were measured and recorded as peak heights. The organic fraction was calculated as the difference between total Hg and inorganic Hg.

2.3 *Reagents and solutions*

All chemicals were of analytical-reagent grade. They included: deionised high purity water (18.2 MΩ cm^{-1}) obtained using a Milli-Q system (Millipore, Bedford, MA); tetramethylammonium hydroxide (TMAH) in methanol – 25.0% (w/v) aqueous solution (Aldrich; 33, 490-1 – Milwaukee, USA); hydrochloric acid (1.00317; Merck Darmstadt, Germany);

potassium permanganate (1.05084; Merck Darmstadt, Germany); L-cysteine hydrochloride monohydrate (Fluka 30130 Buchs, Switzerland); tin chloride (33280; Synth, São Paulo, Brazil); mercury chloride (104419; Merck Darmstadt, Germany); methylmercury chloride (Aldrich 10567EC – Milwaukee, USA); pure argon – 99.98% (Air Liquid – São Paulo, Brazil).

Inorganic mercury and methylmercury working standard solutions were prepared daily from stock solutions of 1,000 mg L^{-1} Hg^{2+} and 1,000 mg L^{-1} CH_3Hg^+, respectively, in 2.0% (w/v) TMAH; they were stored in the dark at 4°C prior to use. Total and inorganic Hg fish sample concentrations were calculated using the Hg^{2+} calibration curves.

The 0.5 and 1.0% (w/v) $SnCl_2$ in 10% (v/v) HCl solutions, the 0.1% (w/v) $KMnO_4$ and the 0.1% (w/v) L-cysteine solutions were daily prepared. Both $SnCl_2$ solutions were purged with argon at 50 mL min^{-1} for 30 minutes before use. The 0.1% (w/v) $KMnO_4$ solution was prepared in deionised water and stored in a dark flask, protected from light to minimise photodecomposition.

2.4 *Sample preparation*

The fish samples were prepared in a vertical flow, clean air, workstation. Fifty mg of blue-shark (*Prionace glauca*) lyophilised muscular tissue were accurately weighed and transferred to a 50 mL *Falcon* polypropylene tube; the sample was then dissolved by the addition of 4.0 mL of 25% (w/v) TMAH solution [13,14,20,24]. TMAH solutions of 6.25, 12.50, 18.75 and 25% (w/v) were compared; 25% (w/v) was found to be the optimum TMAH concentration for this method. The weighed *Falcon* tubes were transferred to an ultrasound bath (VWR Aquasonic 75D) for 15 min, at room temperature [36]. Blank solutions and certified reference samples were processed by using a similar procedure. The sample solution was made up to 50 mL with deionised water.

Analytical accuracy was assessed by analysing sub-samples of lyophilised dogfish muscular tissue, a Certified Reference Material (CRM: DORM-2, National Research Council of Canada), alongside experimental fish samples. The certified Hg values for CRM DORM-2 are 4.64 ± 0.26 mg kg^{-1} Hg and 4.47 ± 0.32 mg kg^{-1} CH_3Hg.

3. Results and discussion

3.1 *The FI manifold*

The reaction coils length (Figure 1: L_1 and L_2) and reagent flow rates (Figure 1: R_1 and R_2) were based on Tao *et al*. [20]. In addition, recommendations by Martelli *et al*. [34] and Reis *et al*. [35] to minimise sample dispersion and to increase Hg^0 yield were taken into account when designing the FI system. For total Hg determination L_1 should be selected in order to allow enough time for Hg oxidation from sample organic matter. In the case of inorganic Hg, the coil needs to be long enough to provide complete release of Hg^{2+}, from sample matrix, by the L-cysteine. Optimum conditions, satisfying these requirements, were observed when coil L_1 was 20 cm, L_2 was 30 cm and the reagents flow rates were 0.7 mL min^{-1} (Figure 1).

Higher reagent flow rates led to a decrease in peak height due to sample dilution [35]. Figure 2 shows the influence of the sample carrier stream flow rates on sensitivity, where the highest peak height for a 2.0 μg L^{-1} CH_3Hg standard solution was achieved at 1.8 mL min^{-1}. However, the slightly lower sensitivity at 2.4 mL min^{-1} flow rate was

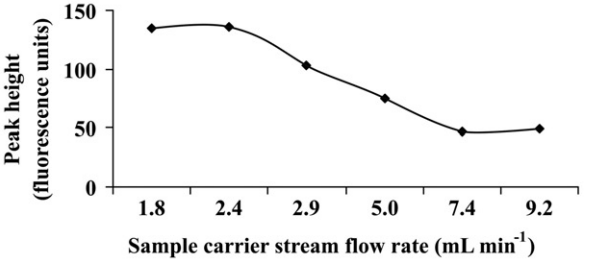

Figure 2. The effect of sample carrier stream (HCl 0.1 mol L^{-1}) flow rate on Hg peak height (data based on triplicate measurements of a 2.0 μg L^{-1} CH_3Hg^+ standard solution signal).

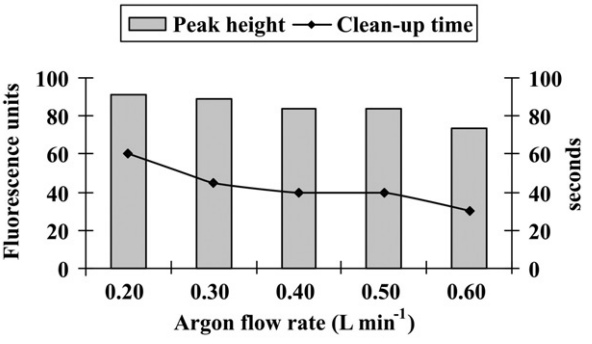

Figure 3. The effect of carrier gas flow rate (mL min^{-1} Ar) on Hg peak height and clean-up time (data based on triplicate measurements of a 2.0 μg L^{-1} CH_3Hg^+ standard solution signal).

selected when sampling rate was also considered. It is important that the sample carrier stream does not decrease the pH to a value lower than 12.0 before $SnCl_2$ joins the FI analytical path because the reaction between Hg^{2+} and L-cysteine is strongly pH dependent [14]. The sample carrier was selected as 0.1 mol L^{-1} HCl [14].

Higher peak heights were observed when sample volume was increased. A 1,000 μL sample loop was selected. Decreases in peak height of 42.3 and 27.9% were observed when 500 and 750 μL sample loops were used, respectively.

The argon carrier gas flow rate affects the Hg^0 vapour dilution and the clean-up time of the system, mainly between the GLS and detector. Faster clean-up speed was observed as Ar flow rate was increased, while higher peak heights were obtained as Ar flow rate decreases (Figure 3). An argon flow rate of 0.40 L min^{-1} was selected after consideration of sample throughput time versus system clean-up times; this achieved a sampling rate of 51 samples h^{-1} with a clean-up time of ~40 seconds and a near maximum peak height.

3.2 *Sample preparation*

For all TMAH concentrations studied, the *Falcon* polypropylene tubes containing sample solutions were placed in an ultrasound bath for 15 min because sonication was found to improve the sample solubilisation process [36]. Longer times did not increase the analyte

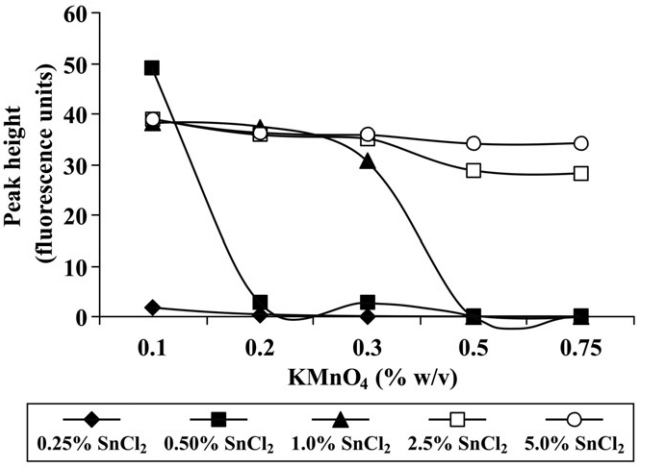

Figure 4. Effect of $KMnO_4$ and $SnCl_2$ concentrations (% w/v) on mercury peak height. The data are related to a blue-shark sample. Signals for triplicate measurements.

extraction for this study. The best Hg recovery (99.1%, total Hg) was obtained when a 25.00% (w/v) TMAH solution was used. For TMAH concentrations of 6.25, 12.50 and 18.75% (w/v), recoveries for total Hg were 68.0, 90.6 and 95.6%, respectively.

3.3 *Total mercury*

Sample solutions were diluted to reduce organic matter concentrations before sample introduction in the FI manifold, enhancing the on-line $KMnO_4$ oxidation conditions for the total Hg determination [21–23]. Recoveries of 71.3, 85.0 and 99.1% were achieved when sample solutions were diluted 1, 3 and 5 times, respectively. In this study a five-fold sample dilution, combined with a 0.1% (w/v) $KMnO_4$ solution was found to provide optimum organic Hg species oxidation and did not interfere with the subsequent $SnCl_2$ mercury reduction process [37]. This approach eliminated the need for on-line microwave heating to improve oxidation [22,38], or, for the injection of microwave-assisted sample solution into the FI manifold for further on-line oxidation [39].

It was observed that when a 0.25% (w/v) $SnCl_2$ solution was used in combination with $KMnO_4$ solutions in a wide range of concentrations (0.1 to 0.75% w/v), no signals were detected (Figure 4). Well defined Hg peak heights were observed by using 1.0, 2.5 and 5.0% (w/v) $SnCl_2$ solutions plus a 0.1% (w/v) $KMnO_4$ solution. Low peak heights were observed when $KMnO_4$ solution was $>0.5\%$ (w/v) as it impairs the Hg reduction in the FI system. Peak heights were similar for 2.5 and 5.0% (w/v) $SnCl_2$ solutions, but optimum analytical conditions were observed when using a 0.5% (w/v) $SnCl_2$ reducing agent in tandem with a 0.1% (w/v) $KMnO_4$ oxidising agent. Results for total Hg in dogfish (DORM2) were within the certified standard deviation limit using these analytical conditions (Table 1).

The use of $KMnO_4$ oxidising agent with an $NaBH_4$ reductant, as recommended elsewhere [20–24], was investigated and abandoned due to an excessive foam evolving in the GLS; this led to a reduction in Hg vapour generation, even when the sample solutions were diluted five-fold. The Hg reduction by $SnCl_2$ produced no foam; therefore an anti-foaming agent was not required.

Table 1. Total and organic mercury ($mg\,kg^{-1}$) in dogfish (CRM DORM-2; NRCC), obtained using the proposed method (triplicate analysis).

Certified reference material	Total Hg ($mg\,kg^{-1}$)	Organic Hg ($mg\,kg^{-1}$)
Certified values	4.64 ± 0.26	4.47 ± 0.32
Obtained	4.60 ± 0.05	4.20 ± 0.07
Recovered (%)	99.1	93.9

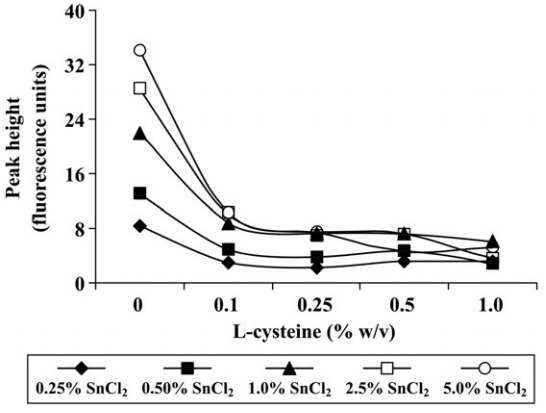

Figure 5. The effect of L-cysteine and $SnCl_2$ concentrations (% w/v) on mercury peak height. The data are for certified reference dogfish (CRM DORM-2; NRCC). Signals for triplicate measurements.

Optimum conditions for total Hg were obtained when sample solutions were diluted five-fold and injected into the FI system followed by sequential reactions with a 0.1% (w/v) $KMnO_4$ oxidising agent and a 0.5% (w/v) $SnCl_2$ reducing agent. This avoided having to use a stronger reducing agent to enhance elemental Hg^0 generation [37].

3.4 *Inorganic mercury*

The role of cysteine on the determination of inorganic Hg in biological samples has been widely discussed in the literature [10,14–19]. Cysteine releases Hg^{2+} from ligands in organic matter, which can then be reduced by $SnCl_2$ [14]. It also avoids the reduction of organic Hg compounds, as organic Hg has a high affinity to L-cysteine thiolic groups [16]. The Hg^{2+} complexation to –SH radicals is fast, without the need of any incubating time, or use of an excessive amount of reagent [14,17].

It is difficult to distinguish inorganic Hg from the total Hg. Several papers have reported the non reduction of the organic Hg to elemental Hg by the $SnCl_2$, and that only a selective reduction of the inorganic Hg can be achieved in the presence of this reductant [25,31]. In the absence of L-cysteine, higher peak heights were obtained as the $SnCl_2$ solution concentrations increased (Figure 5). Furthermore, partial reduction of organic Hg by $SnCl_2$ occurred [41] with a slower reduction rate of organic than inorganic mercury. This clearly demonstrates the role of the complexant agent (L-cysteine), on Hg speciation.

L-cysteine concentrations of 0.1, 0.25, 0.5 and 1.0% (w/v) were tested (Figure 5), and, as no differences in peak height were observed in the selected range, a 0.1% (w/v) L-cysteine concentration was selected.

When $SnCl_2$ concentrations of 1.0, 2.5 and 5.0% (w/v) were used, no significant difference in peak heights for inorganic Hg were observed. Therefore, a 1.0% $SnCl_2$ (w/v) solution was selected (Figure 5). By using a 1.0% (w/v) $SnCl_2$ plus a 0.1% (w/v) L-cysteine solutions, a high percentage recovery for inorganic Hg analysis of CRM-DORM-2 was achieved (Table 1); the organic Hg concentration was calculated by the difference between the total and inorganic Hg.

Inorganic Hg constitutes around 5% of the total Hg concentration in fish muscular tissue extracts [40]. Therefore, the sample solution should not be diluted, as this can compromise the analytical method sensitivity.

3.5 *Analytical figures of merit*

By using the proposed method for routine determinations of Hg concentrations in blue-shark (*Prionace glauca*) muscular tissue samples ($n = 27$), we were able to quantify inorganic Hg concentrations ranging between 0.008 and 0.033 mg kg^{-1}, and total Hg concentrations ranging from 0.46 to 2.40 mg kg^{-1}.

Percentage Hg recoveries for the dogfish certified reference sample (CRM-DORM-2), are presented in Table 1. Results were in agreement with the certified values, at a 95% confidence level. The repeatability, evaluated by the relative standard deviation (RSD) for a 1.0 μg L^{-1} CH_3Hg standard solution ($n = 20$) was 1.1%, and 1.3% for a 1.0 μg L^{-1} Hg^{2+} standard solution ($n = 20$).

For total Hg, the analytical curve was obtained in the 0.25 to 2.00 μg L^{-1} range; for inorganic Hg, the standard solutions concentrations ranged from 0.10 to 0.50 μg L^{-1}. Analytical curves obtained using these Hg standard solutions obeyed the linear equations $P = 42.1\,C + 4.125$ (correlation coefficient $r^2 = 0.999$) and $P = 11.80\,C + 0.400$ ($r^2 = 0.999$) for total and inorganic Hg respectively, where P is peak height signal and C is the Hg concentration in μg L^{-1}. The limit of detection (LOD) were 3.7 ng g^{-1} (or 0.059 μg L^{-1}) for total Hg and 4.3 ng g^{-1} (or 0.070 μg L^{-1}) for inorganic Hg. The limit of quantification (LOQ) was 4.7 and 8.1 ng g^{-1} for total and inorganic Hg, respectively.

Although Hg losses due to Hg^0 generation in alkaline medium and Hg species converting like inorganic methylation during sample preparation have been reported [29,31–33], recoveries for the dogfish certified reference indicated minimal Hg losses during the analytical procedure (Table 1). Sample solutions ($n = 3$) re-analysed after seven days storage period at 4°C were stable and produced similar results, at 95% of confidence.

3.6 *Comparison with other methods*

In general, considering reagent consumption and simplicity, results bring an improvement for the on-line Hg determination in fish samples in comparison with a range of published methods [14,20,22,24,38,39]. Table 2 summarises the advantages and disadvantages of these, and other published Hg methods.

The FI manifold is simple and robust. The proposed procedure is sensitive and was able to detect Hg concentrations in fish samples at 4 ng g^{-1} level. This feature could be

Table 2. Analytical parameters for the determination of total and inorganic Hg in biological samples by flow injection cold vapour atomic spectrometry: a comparison of published methods and the present work, where C = (% w/v); F = flow rate ($mL\,min^{-1}$); S = sample carrier stream (HNO_3 or HCl mol L^{-1}); P = number of peristaltic pumps.

		Total mercury						Inorganic mercury			
Parameters		[20]	[22]	[24]	[38]	[39]	Present work	[14]	[38]	[39]	Present work
Sample		Fish	Blood	Blood	Fish	Fish	**Fish**	Fish	Fish	Fish	**Fish**
Reagents											
$KMnO_4$	C	0.2	0.05	0.2	2.0*		**0.1**	–	2.0*	–	–
	F	3.0	5.0	5.0	2.0		**0.7**	–	2.0	–	–
$NaBH_4$	C	0.2	0.05	0.2	0.4**	0.75	–	–	0.4**	10^{-4}	–
	F	3.0	5.0	5.0	3.0	6.0	–	–	3.0	6.0	–
$SnCl_2$	C	–	–	–	–	–	**0.5**	1.0	–	–	**1.0**
	F	–	–	–	–	–	**0.7**	3	–	–	**0.7**
L-cysteine	C	–	–	–	–	–	–	0.5	–	–	**0.1**
	F	–	–	–	–	–	–	3.0	–	–	**0.7**
S	C	0.1	0.0	0.1	4.0	3.0	**0.1**	0.1	4.0	3.0	**0.1**
	F	10.0	9.0	14.0	4.0	9.5	**2.4**	10.0	4.0	5.5	**2.4**
Figures of merit											
Sample volume (μL)		500	500	500	–	500	**1,000**	500	–	500	**1,000**
Sampling rate (h^{-1})		100	45	38	102	28	**51**	100	102	28	**51**
RSD (%)		1.3	6.0	6.0	3.2	1.5	**1.1**	2.0	2.4	1.5	**1.3**
CRM Recovery (%)		97.6	–	96.1	98.7	97.1	**99.1**	129.4	102.9	102.9	**93.9**
LOD ($\mu g\,L^{-1}$)		0.100	0.100	0.170	0.002	0.024	**0.059**	0.200	0.002	0.004	**0.070**
Additional requirements											
Antifoam reagent		Yes	Yes	Yes	No	No	**No**	Yes	No	No	**No**
P		02	02	3	01	02	**01**	02	01	02	**01**
*Heating***		No	Yes	No	Yes	Yes	**No**	No	Yes	Yes	**No**

*$K_2S_2O_8$; **KBH_4; ***on-line microwave or microwave-assisted extraction.

considered as an advantage in comparison with other low LOD methods that require complex sample preparation steps, such as on-line microwave sample heating [38] and a microwave-assisted sample extration to enhance the organomercury on-line oxidation [39]. The RSD achieved is in the same average of other published methods. An average sample throughput time was obtained, but a higher sampling loop (sample volume) was used (Table 2).

The FI manifold for this method is simple because it only needs one peristaltic pump; the same manifold, and a sole reductant, can be used for both, total and inorganic Hg analysis. The $SnCl_2$ solution is simple to purify, cheaper than $NaBH_4$, and not so reactive. When $NaBH_4$ is used, foam is produced in the GLS requiring the introduction of an anti-foaming agent.

Considering the rapid sample preparation procedure, which can be carried out at room temperature, the whole analytical methodology provides a fast Hg determination. Hence, it can be used in routine Hg monitoring in fish samples. Coupling the FI manifold to an Hg analyser, the proposed method can be used by analysts working in laboratories which do not have chromatographic facilities.

4. Conclusions

This work presents a simple and robust analytical method for the determination of total and inorganic Hg in fish tissue. The sample solubilisation with TMAH at room temperature and the on-line oxidation of organomercury species for a further reduction within the FI system reduces opportunities for Hg losses and sample contamination. Mercury recoveries obtained for dogfish CRM sub-samples indicate that this is a reliable and accurate method for assessing Hg speciation in fish samples. Compared with other published methods, this method reduces reagent consumption because of the simple FI manifold system. It has proved to be a reliable and a practical method for the routine monitoring of Hg concentrations in fish samples.

Acknowledgements

The authors thank Dr Elias A.G. Zagatto for his thorough review and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for providing the scholarship to G.G.A. Carvalho (Process No. 110751/2005-1).

References

[1] M.M. Storelli, E. Ceci, A. Storelli, and G.O. Marcotrigiano, Mar. Pollut. Bull. **46**, 1035 (2003).
[2] M.C. Bisinoti and W.F. Jardim, Quim. Nova **27**, 593 (2004).
[3] A.I. Cabañero, Y. Madrid, and C. Câmara, J. Agr. Food Chem. **53**, 2125 (2005).
[4] N. Domi, J.M. Bouquegneau, and K. Das, Mar. Environ. Res. **60**, 551 (2005).
[5] D.W. Boening, Chemosphere **40**, 1335 (2000).
[6] P. Grandjean, P. Weihe, R.F. White, F. Debes, S. Araki, K. Yokoyama, K. Murata, N. Sorensen, R. Dahl, and P.J. Jorgensen, Neurotixicol. Teratol. **19**, 417 (1997).
[7] R. Puk and J.H. Weber, Appl. Organometal. Chem. **8**, 293 (1994).
[8] C.M. Davidson, R.P. Thomas, S.E. McVey, R. Perala, D. Littlejohn, and A.M. Ure, Anal. Chim. Acta **291**, 277 (1994).
[9] J. Gailer, Coord. Chem. Rev. **251**, 234 (2007).
[10] E. Bramanti, C. Lomonte, M. Onor, R. Zamboni, A. D'Ulivo, and G. Raspi, Talanta **66**, 762 (2005).
[11] H. Morita, S. Tanaka, and S. Shimomura, Spectrochim. Acta Part B **50**, 69 (1995).
[12] L.E. Kaercher, F. Goldschmidt, J.N.G. Paniz, É.M.M. Flores, and V.L. Dressler, Spectrochim. Acta Part B **60**, 705 (2005).
[13] D.P. Torres, M.A. Vieira, A.S. Ribeiro, and A.J. Curtius, J. Anal. At. Spectrom. **20**, 289 (2005).
[14] G. Tao, S.N. Willie, and R.E. Sturgeon, J. Anal. At. Spectrom. **14**, 1929 (1999).
[15] R.S. Daniels and D.C. Wigfield, Sci. Total Environ. **89**, 325 (1989).
[16] C. Sarzanini, G. Sacchero, M. Aceto, O. Abollino, and E. Mentasti, Anal. Chim. Acta **284**, 661 (1994).
[17] E. Bramanti, A. D'Ulivo, L. Lampugnani, G. Raspi, and R. Zamboni, J. Anal. At. Spectrom. **14**, 179 (1999).
[18] U. Gill, L. Bigras, and H. Schwartz, Chemosphere **56**, 1097 (2004).
[19] S.C. Hight and J. Cheng, Anal. Chim. Acta **567**, 160 (2006).
[20] G. Tao, S.N. Willie, and R.E. Sturgeon, The Analyst **123**, 1215 (1998).
[21] T. Guo, J. Baasner, M. Gradl, and A. Kistner, Anal. Chim. Acta **320**, 171 (1996).
[22] T. Guo and J. Baasner, Talanta **40**, 1927 (1993).
[23] T. Guo and J. Baasner, Anal. Chim. Acta **278**, 189 (1993).
[24] F. Barbosa Jr, C.D. Palmer, F.J. Krug, and P.J. Parsons, J. Anal. At. Spectrom. **19**, 1000 (2004).

[25] C.J. Park and H. Do, J. Anal. At. Spectrom. **23**, 997 (2008).
[26] D. Pozebon, V.L. Dressler, and A.J. Curtius, J. Anal. At. Spectrom. **13**, 1101 (1998).
[27] H. Matusiewicz and B. Golik, Microchem. J. **76**, 23 (2004).
[28] J.L. Rodrigues, B.L. Batista, J.A. Nunes, S.S. de Souza, and F. Barbosa Jr, J. Anal. At. Spectrom. **23**, 992 (2008).
[29] A.I. Cabañero-Ortiz, Y.M. Albarrán, and C. Cámara-Rica, J. Anal. At. Spectrom. **17**, 1595 (2002).
[30] M.C. Paul, R.F. Toia, and E.I. von Nagy-Felsobuki, Spectrochim. Acta Part B **58**, 1687 (2003).
[31] I. Serafimovski, I. Karadjova, T. Stafilov and J. Cvetkovic, Microchem. J. **89**, 42 (2008).
[32] L.H. Reyes, G.M.M. Rahman, T. Fahrenholz, and H.M.S. Kingston, Anal. Bioanal. Chem. **390**, 2123 (2008).
[33] E.J. Santos, A.B. Herrmann, V.L.A. Frescura, R.E. Sturgeon, and A.J. Curtius, J. Braz. Chem. Soc. **19**, 929 (2008).
[34] P.B. Martelli, B.F. Reis, M. Korn, and I.A. Rufini, J. Braz. Chem. Soc. **08**, 479 (1997).
[35] B.F. Reis, M.F. Giné, and E.A.M. Kronka, Quim. Nova **12**, 82 (1989).
[36] D. Santos Jr, F.J. Krug, M.G. Pereira, and M. Korn, Appl. Spec. Rev. **41**, 305 (2006).
[37] M. Gallignani, H. Bahsas, M.R. Brunetto, M. Burguera, J.L. Burguera, and Y. Petit de Pena, Anal. Chim. Acta **369**, 57 (1998).
[38] L.J. Shao, W.E. Gan, and Q.D. Su, Anal. Chim. Acta **562**, 128 (2006).
[39] S. Rio-Segade and J.F. Tyson, Spectrochim. Acta Part B **58**, 797 (2003).
[40] T. Agusa, T. Kunito, G. Yasunaga, H. Iwata, A. Subramanian, A. Ismail, and S. Tanabe, Mar. Pollut. Bull. **51**, 896 (2005).
[41] J.L. Capelo, G.M. Rivas, L.G. Oliveira, C. Vilhena, A.C. Santos, T. Valada, M. Galesio, P. Oliveira, M.D.R. Gomes da Silva, E.M. Gaspar, S. Alves, C. Fernansdez, and C. Vaz, Talanta **68**, 813 (2006).