2003

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SR Segade

JF Tyson

Determination of inorganic mercury and total mercury in biological and environmental samples by flow injection-cold vapor-atomic absorption spectrometry using sodium borohydride as the sole reducing agent

Susana Río Segade[a,*], Julian F. Tyson[b]

[a]*Departamento de Química Analítica y Alimentaria, Universidad de Vigo, Facultad de Ciencias (Química), Lagoas-Marcosende s/n 36200, Vigo, Spain*
[b]*Department of Chemistry, University of Massachusetts, Amherst, MA 01003, USA*

Received 19 November 2002; accepted 4 February 2003

Abstract

A simple, fast, precise and accurate method to determine inorganic mercury and total mercury in biological and environmental samples was developed. The optimized flow-injection mercury system permitted the separate determination of inorganic mercury and total mercury using sodium borohydride as reducing agent. Inorganic mercury was selectively determined after reduction with 10^{-4}% w/v sodium borohydride, while total mercury was determined after reduction with 0.75% w/v sodium borohydride. The calibration graphs were linear up to 30 ng ml^{-1}. The detection limits of the method based on three times the standard deviation of the blank were 24 and 3.9 ng l^{-1} for total mercury and inorganic mercury determination, respectively. The relative standard deviation was less than 1.5% for a 10 ng ml^{-1} mercury standard. As a means of checking method performance, deionized water and pond water samples were spiked with methylmercury and inorganic mercury; quantitative recovery for total mercury and inorganic mercury was obtained. The accuracy of the method was verified by analyzing alkaline and acid extracts of five biological and sediment reference materials. Microwave-assisted extraction procedures resulted in higher concentrations of recovered mercury species, lower matrix interference with mercury determination and less time involved in sample treatment than conventional extraction procedures. The standard addition method was only needed for calibration when biological samples were analyzed. The detection limits were in the range of 1.2–19 and 6.6–18 ng g^{-1} in biological and sediment samples for inorganic mercury and total mercury determination, respectively.

Keywords: Flow injection; Inorganic mercury; Total mercury; Water; Biological samples; Sediment; Microwave-assisted extraction

*Corresponding author. Tel.: +34-986-814083; fax: +34-986-812556.
E-mail address: srio@uvigo.es (S. Río Segade).

1. Introduction

Mercury has been recognized as one of the most toxic heavy metals present in the environment. However, total mercury concentration yields little information about its toxicological and biogeochemical behavior, which depends on the specific chemical form [1]. It is well known that methylmercury is more toxic than inorganic mercury [2]. The anthropogenic sources of methylmercury are usually rare, but it is naturally formed in sediments by bacterial methylation of inorganic mercury [3]. Methylmercury can then be bioaccumulated and biomagnified in the food chain [4]. Hence, the consumption of contaminated seafood, especially predatory fish and marine mammals, with methylmercury represents a potential hazard to human health [4]. Furthermore, the analysis of sediments and fish tissues permits monitoring of mercury levels in water, since mercury present in contaminated waters is accumulated in both environmental compartments.

As a consequence, not only total mercury determination, but also methylmercury determination, is needed in order to know the toxicological and environmental impact of mercury. The most commonly used technique for mercury determination in biological and environmental samples is cold vapor-atomic absorption spectrometry (CV-AAS) due to its analytical abilities [5]. Some methods have been published on mercury speciation by CV-AAS without previous chromatographic separation, based on either the use of several reducing agents with different reducing power, such as sodium borohydride and stannous chloride, or the possible oxidation of organomercury species to inorganic mercury previous to the reduction to elemental mercury.

In the first method, inorganic mercury is selectively determined using stannous chloride in acid medium as reducing agent due to the inability to reduce organomercury compounds [6–8]. Furthermore, total mercury determinations are carried out using sodium borohydride due to its power to reduce both inorganic and organic mercury species when the sensitivity of the species does not differ significantly [8]. However, some authors have obtained different sensitivity for methylmercury and inorganic mercury in several matrices when sodium borohydride is used as reducing agent for total mercury determination [8–13]. The formation of methylmercury hydride (MeHgH) instead of elemental mercury may be the cause of the different behavior of both species [14–17]. A paper has recently been published based on the use of sodium borohydride for inorganic mercury determinations [18]. This problem is solved by the oxidation of organomercury compounds to inorganic mercury, using combinations of strong acids (hydrochloric, sulfuric and nitric acids), oxidants (hydrogen peroxide, potassium permanganate, potassium dichromate, potassium persulfate, potassium bromide–potassium bromate), elevated temperatures, ultraviolet irradiation, microwave exposure and sonolysis, previous to reduction to elemental mercury [9,18,19]. Other methods are also based on selective reduction of inorganic mercury using stannous chloride in acid medium as reducing agent and the determination of total mercury with the same reducing agent after the oxidation of organomercury compounds as mentioned above [9,10,20–22]. Organomercury species, mainly methylmercury, are determined by difference between total and inorganic mercury concentrations.

Some workers report that sodium borohydride allows the determination of total mercury without prior oxidative treatment in batch systems [23]. The lower concentration of sodium borohydride used and the shorter reaction time required in flow injection systems (FI) compared to those involved in batch systems could be the cause of the low sensitivity obtained for methylmercury in relation to inorganic mercury sensitivity in FI-CV-AAS systems [8–13,18,19].

In this work, a new methodology is proposed for mercury speciation that permits differentiation between inorganic mercury and total mercury in a flow injection mercury system (FIMS) using sodium borohydride as the sole reducing agent. Inorganic mercury was selectively determined with a low concentration of sodium borohydride as the reducing agent. Similar sensitivities were obtained for methylmercury and inorganic mercury when high concentrations of sodium borohydride were used for total mercury determination, avoiding the

need for oxidative treatment. Mercury speciation solely based on the use of different concentrations of sodium borohydride has not been previously reported in the literature. All FI parameters were optimized and the FIMS was characterized in relation to its analytical properties. This methodology was then applied to mercury speciation in water samples and validated by the analysis of two fish-tissue certified reference materials (CRMs), two sediment CRMs and one fish-tissue control sample. Furthermore, the effect of different extraction methods on the precision, accuracy and sensitivity of inorganic mercury and total mercury determinations was studied in the solid samples mentioned above. The combination of the proposed FI methodology with microwave-assisted extraction (MAE) methods permitted the rapid and simple determination of inorganic mercury and total mercury in sediments and fish tissues. Methylmercury could be determined by difference between total mercury and inorganic mercury concentrations.

2. Experimental

2.1. Instrumentation

A Perkin Elmer flow-injection mercury system (FIMS) model 400 (Überlingen, Germany) equipped with a flow injection analysis system (FIAS) and an autosampler model AS-91 was used for all mercury determinations. This system consisted of two peristaltic pumps (P_1 and P_2), a flow meter, a cylindrical gas–liquid separator partially filled with glass beads, a six-way injection valve equipped with a sample loop, and a quartz cell (25 cm in length with quartz windows). The sample was injected into the system and transported in an acid carrier to the chemifold, where it was mixed with the reducing agent along a reduction coil (R_1). Mercury vapor was then purged from the liquid solution along a stripping coil (R_2) with an argon stream before its entrance to the gas–liquid separator, and was then swept into the quartz cell.

The peristaltic pumps, injection time and data acquisition were controlled through Perkin Elmer AAWinLab atomic absorption spectroscopy software (Norwalk, CT, USA). The FIAS program used for all mercury determinations is shown in Table 1. The sample loop was filled by means of pump P_2. The acid carrier, reducing agent and waste solution from the gas–liquid separator were pumped using peristaltic pump P_2 through Tygon tubes, and the waste solution from the injection valve was pumped with a peristaltic pump P_1 through Tygon tubing. The manifold tubing was made of 1.0-mm-i.d. Teflon (FEP). An integration time of 20 s and peak height measurement mode were used.

Table 1
FIAS 400 program for mercury determination

Step	Time (s)	P_1 speed (rev./min)	P_2 speed (rev./min)	Valve position	Read
Prefill	15	100	120	Fill	No
1	10	100	120	Fill	No
2	15	0	75/120[a]	Inject	Yes
3	0			Fill	No

[a] $P_2 = 75$ for inorganic mercury determination and $P_2 = 120$ for total mercury determination.

A Fisher Scientific magnetic stirrer (Fair Lawn, NJ, USA), a CEM microwave oven model MDS-81D (Matthews, NC, USA) with 630-W maximum output, a Fisher Scientific centrifuge and a Fisher Scientific pH meter model 915 were used for sample preparation.

2.2. Reagents, standards, samples and reference materials

All solutions were prepared in deionized water produced by a Barnstead E-Pure system and the chemicals used were of analytical reagent grade. The 10^{-4} and 0.75% w/v sodium borohydride reducing agent for inorganic mercury and total mercury determination was prepared daily by dissolution of the appropriate amount of the solid reagent (Alfa Aesar, Ward Hill, MA, USA) in a 0.001 and 1.0% w/v sodium hydroxide solution, respectively. The carrier was 3.0% v/v hydrochloric acid. The stock standard solution of mercury nitrate (1000 mg l^{-1}) was supplied by Alfa Aesar. The stock standard solution of methylmercury chloride (100 mg l^{-1}) was prepared by dissolving the appropriate amount of the solid

reagent from Strem Chemicals (Newburyport, MA, USA) in a minimum volume of methanol and diluting it to volume with deionized water. The working standard solutions for each individual mercury species were prepared daily by appropriately diluting the 10 mg l^{-1} (as Hg) standard solutions, prepared weekly, with the acid carrier. All standards were stored at 4 °C away from light before use.

The pond water sample was filtered through 0.45-μm membranes from Millipore (Bedford, MA, USA). Two biological reference materials (NRC DOLT-2 dogfish liver and NRC DORM-2 dogfish muscle) with different certified contents of methylmercury and total mercury, both obtained from the National Research Council of Canada (NRC, Ottawa, Ontario, Canada), two sediment reference materials (NIST 1944 waterway sediment and NIST 2704 river sediment) with different certified contents of total mercury, both obtained from the National Institute of Standards and Technology (NIST, Washington, USA) and one biological control sample (QC 91-LH1 Whale liver) with known contents of methylmercury and total mercury, obtained from the National Institute of Standards and Technology (NIST), were used to validate the proposed method.

2.3. Sample preparation

Methylmercury and inorganic mercury were extracted from biological samples and sediments by four different treatments: conventional extraction in alkaline medium; conventional extraction in acid medium; microwave-assisted extraction in alkaline medium; and microwave-assisted extraction in acid medium. Blanks were subjected to the same procedures as samples.

2.3.1. Conventional extraction procedure

A 0.5-g portion of dry reference material or 1.5 g of wet control sample were accurately weighed in glass tubes and suspended in 5 ml of extractant. After the tubes were capped, the suspension was magnetically stirred for 3 h and centrifuged at 5000 rev./min for 5 min. The supernatant liquid was decanted and adjusted to the same pH as the acid carrier. The extract was then transferred into a 50-ml calibrated flask and diluted to volume with deionized water. The extractants used were 25% w/v tetramethylammonium hydroxide and 6 mol l^{-1} hydrochloric acid. The pH was then adjusted to the corresponding value with hydrochloric acid and sodium hydroxide, respectively.

2.3.2. Microwave-assisted extraction procedure

A sample portion of 0.5 g of dry reference material or 1.5 g of wet control sample was accurately weighed in glass tubes and 5 ml of extractant was added. The tubes were carefully capped. After the suspension was homogenized by magnetic stirring, it was exposed to microwave irradiation at 60 W for 3 min [15,24]. The digest was allowed to cool to ambient temperature. The resulting solution was then centrifuged at 5000 rev./min for 5 min and the liquid phase was adjusted to the same pH as the acid carrier. The extract was transferred into a 50-ml calibrated flask and diluted to volume with deionized water. The extractants used were 25% w/v tetramethylammonium hydroxide and 6 mol l^{-1} hydrochloric acid. The pH was then adjusted to the corresponding value with hydrochloric acid and sodium hydroxide, respectively.

3. Results and discussion

3.1. Optimization of the FIMS parameters

The effect of several FIMS parameters, such as sodium borohydride and sodium hydroxide concentrations in the reducing agent, hydrochloric acid concentration in the carrier, reducing agent and acid carrier flow-rates, argon flow rate, length of the reaction coils and sample volume, on the absorbance for methylmercury and inorganic mercury was studied in order to achieve adequate experimental conditions to obtain either similar sensitivity for both mercury species or negligible absorbance for methylmercury. The range studied for these parameters is shown in Table 2. Standard solutions (10 ng ml^{-1} as Hg) of each individual mercury species in the acid carrier were used for the optimization.

Although sodium borohydride is the most widely used reducing agent for total mercury determi-

Table 2
Range and optimum value of FIMS parameters for inorganic mercury and total mercury determination

Parameter	Range studied	Optimum value	
		Hg-I determination	Hg-T determination
$NaBH_4$ concentration (% w/v)	10^{-4}–0.75	10^{-4}	0.75
NaOH concentration (% w/v)	10^{-3}–1.0	10^{-3}	1.0
Reducing agent flow rate (ml min^{-1})	3.0–6.5	4.0	6.5
HCl concentration (% v/v)	3×10^{-3}–4.0	3.0	3.0
Acid carrier flow rate (ml min^{-1})	4.5–9.5	5.5	9.5
Argon flow rate (ml min^{-1})	40–200	75	40
Length of the reduction coil R_1 (cm)	5.0–150	5.0	5.0
Length of the stripping coil R_2 (cm)	15–150	15	15
Sample volume (μl)	50–1000	500	500

nation, the influence of its concentration on the cold vapor generation was investigated. The absorbance signal for methylmercury increased with sodium borohydride concentration from 0.01 to 0.75% w/v. Sodium borohydride concentrations higher than 0.75% w/v could not be used due to the intensive reduction reaction. However, the absorbance signal for methylmercury was less than 0.007 when sodium borohydride concentrations less than 0.01% w/v were employed. The maximum absorbance for inorganic mercury was obtained using sodium borohydride concentrations between 0.15 and 0.50% w/v. This range includes the value recommended by the manufacturer. The decrease in absorbance for inorganic mercury at sodium borohydride concentrations greater than 0.50% w/v is probably due to dilution of the mercury vapor by the hydrogen gas produced. Sodium borohydride concentrations of 10^{-4} and 0.75% w/v were selected for further work to determine selectively inorganic mercury and total mercury, respectively. The relationship between absorbance for both mercury species was 0.02 and 0.36 using sodium borohydride concentrations of 10^{-4} and 0.75% w/v, respectively. A possible explanation for the different behavior of methylmercury and inorganic mercury when reacted with sodium borohydride could be a reduction reaction mechanism. While inorganic mercury was directly reduced to elemental mercury using a wide concentration range of sodium borohydride, methylmercury could generate an intermediate product (MeHgH) using low sodium borohydride concentrations, which would justify the negligible absorbance signal obtained. However, an increase in sodium borohydride concentration seems to improve the elemental mercury generation (Hg^0), as the absorbance signal for methylmercury increased with sodium borohydride concentration.

Both reducing agents were stabilized with different sodium hydroxide concentrations. The sodium hydroxide concentration had no effect on the absorbance for methylmercury and inorganic mercury when sodium borohydride concentration in the reducing agent was 10^{-4}% w/v. Nevertheless, the absorbance for methylmercury increased using sodium hydroxide concentrations higher than 0.30% w/v, while the absorbance for inorganic mercury decreased with increasing sodium hydroxide concentration using 0.75% w/v sodium borohydride. Total mercury determination required a sodium hydroxide concentration of 1.0% w/v due to the need to obtain similar absorbance for both mercury species.

The effect of hydrochloric acid concentration in the carrier on absorbance for methylmercury and inorganic mercury was studied using both reducing agents. It was found that these absorbance values remained virtually constant when the hydrochloric acid concentration was varied and 10^{-4}% w/v sodium borohydride was used as the reducing agent. However, the absorbance values for both mercury species were only similar for hydrochloric acid concentrations $\geq$3.0% v/v for total mercury

Table 3
Determination of inorganic mercury and total mercury in spiked water samples

Mercury added (ng ml^{-1})	Hg-I recovered (ng ml^{-1})	Hg-T recovered (ng ml^{-1})
Deionized water		
10 (Hg^{2+})+2 ($MeHg^{+}$)	10.1±0.1	11.6±0.1
10 (Hg^{2+})+5 ($MeHg^{+}$)	10.2±0.1	14.8±0.1
10 (Hg^{2+})+10 ($MeHg^{+}$)	10.5±0.1	19.5±0.2
10 (Hg^{2+})+20 ($MeHg^{+}$)	10.9±0.1	30.1±0.3
2 (Hg^{2+})+10 ($MeHg^{+}$)	1.93±0.10	11.4±0.3
5 (Hg^{2+})+10 ($MeHg^{+}$)	5.26±0.08	14.8±0.2
10 (Hg^{2+})+10 ($MeHg^{+}$)	10.4±0.1	20.5±0.2
20 (Hg^{2+})+10 ($MeHg^{+}$)	20.3±0.1	30.2±0.3
Pond water		
10 (Hg^{2+})+2 ($MeHg^{+}$)	9.97±0.02	11.7±0.6
10 (Hg^{2+})+5 ($MeHg^{+}$)	10.3±0	15.1±0.4
10 (Hg^{2+})+10 ($MeHg^{+}$)	10.9±0	21.1±0.5
10 (Hg^{2+})+20 ($MeHg^{+}$)	11.3±0.1	29.3±0.4
2 (Hg^{2+})+10 ($MeHg^{+}$)	2.02±0	11.8±0.5
5 (Hg^{2+})+10 ($MeHg^{+}$)	5.13±0.02	15.3±0.5
10 (Hg^{2+})+10 ($MeHg^{+}$)	9.90±0.04	21.1±0.5
20 (Hg^{2+})+10 ($MeHg^{+}$)	21.0±0	29.4±0.5

Results are average value±standard deviation ($n=3$).

determination. Potassium dichromate was added to the acid carrier to obtain a relationship between absorbance for both mercury species of >0.97 for total mercury determination. Nevertheless, the effect observed was not significant.

Other FIMS parameters, which had the same influence on the absorbance for inorganic mercury and methylmercury, were optimized in order to achieve the maximum sensitivity. The maximum values of absorbance for methylmercury and inorganic mercury were obtained using acid carrier and reducing agent flow-rates in the range 5.5–6.5 and 4.0–5.0 ml min^{-1} for 10^{-4}% w/v sodium borohydride or 7.5–9.5 and 6.0–6.5 ml min^{-1} for 0.75% w/v sodium borohydride, respectively. High argon flow-rates seem to decrease the sensitivity for both mercury species because of dilution of the mercury vapor by the gas carrier and reduction of the residence time of the analyte atoms in the quartz cell. Argon flow rates of 75 and 40 ml min^{-1} were selected as optimum values for 10^{-4} and 0.75% w/v sodium borohydride as reducing agent, respectively. Although the reaction coil R_1 promoted mixing between the acid carrier and reducing agent and increased the reduction time, the dispersion was also increased. As a consequence, the absorbance for both mercury species decreased slightly when the length of the reduction coil was increased. This effect was more evident for 0.75% w/v sodium borohydride reducing agent. On the other hand, the length of the stripping coil (R_2), where mercury vapor was purged from the liquid phase, did not affect the absorbance using both reducing agents.

The influence of the sample volume on absorbance for methylmercury and inorganic mercury was examined. Both absorbance values increased with sample volume in the range 50–500 μl, using both reducing agents. Furthermore, absorbance only increased slightly when sample volume was varied from 500 to 1000 μl for 10^{-4}% w/v sodium borohydride as reducing agent. A sample volume of 500 μl was selected for further experiments, as the standard deviation was high when a sample volume of 1000 μl was injected.

3.2. Analytical figures of merit

The analytical performance of the FIMS system was evaluated using the optimum experimental conditions described in Table 2. The system was calibrated with a series of methylmercury and inorganic mercury standards of up to 30 ng ml^{-1}. The equations corresponding to a least-squares regression analysis of calibration curves are as follows:

$$H_T = (1.6\times10^{-2}\pm7.0\times10^{-4})\ [Hg^{2+}] + (2.4\times10^{-3}\pm1.7\times10^{-3})$$

$$H_T = (1.6\times10^{-2}\pm8.5\times10^{-4})\ [MeHg^{+}] + (-1.9\times10^{-3}\pm5.0\times10^{-3})$$

$$H_I = (2.4\times10^{-2}\pm1.3\times10^{-3})\ [Hg^{2+}] + (3.9\times10^{-3}\pm6.0\times10^{-3})$$

where the slopes and the intercepts of the calibration curves are expressed as average value±standard deviation ($n=4$), H_T and H_I are the

peak-height absorbance values for total mercury and inorganic mercury, and [$MeHg^+$] and [Hg^{2+}] are methylmercury and inorganic mercury concentrations (ng ml^{-1}), respectively. As expected, the slopes of the calibration curves for total mercury determination obtained with methylmercury and inorganic mercury standards were not significantly different (t-test, $P=0.05$). Furthermore, the slope of the calibration curve for inorganic mercury determination was greater than that corresponding to total mercury determination. The detection limit based on the amount necessary to yield a net signal equal to three times the standard deviation of the blank was 24 and 3.9 ng l^{-1} for total mercury and inorganic mercury determination, respectively. The relative standard deviation ($n=10$) for a 10 ng ml^{-1} mercury standard was less than 1.5% for both mercury determinations.

It should be noted that these detection limits are lower than those reported for other mercury speciation methods in FI-CV-AAS systems based on: (i) selective determination of inorganic mercury using stannous chloride in acid medium or sodium borohydride as reducing agents, and total mercury determination with the same reducing agent after the oxidation of organomercury compounds; or (ii) selective determination of inorganic mercury using stannous chloride in acid medium as reducing agent and total mercury determination using sodium borohydride reducing agent, with or without oxidation of organomercury species. The detection limits were: 0.12 [18]; 0.10 [10] −0.47 [25]; 0.17 [8]; and 0.45 [19] ng ml^{-1} for inorganic mercury; and 0.11 [18]; 0.10 [10] −0.45 [25]; 0.25 [8]; and 0.14 [19] −0.4 [9] ng ml^{-1} for total mercury; using the following combinations: sodium borohydride/oxidation; stannous chloride/oxidation; stannous chloride/sodium borohydride; and stannous chloride/sodium borohydride/oxidation, respectively.

3.3. Determination of inorganic mercury and total mercury in spiked water samples

The reliability of the proposed method for mercury speciation in water samples was examined by analyzing deionized water and pond water samples spiked with different concentrations (2.0–20 ng ml^{-1}) of methylmercury and inorganic mercury, because these samples did not contain detectable amounts of both mercury species, which are the most prevalent forms of mercury found in environmental waters. The results obtained are shown in Table 3. It can be observed that the recovery values for inorganic mercury and total mercury were higher than 95% in water samples.

3.4. Validation of the method

The proposed method was validated by the analysis of two biological reference materials, two sediment reference materials and one biological control sample. The samples were analyzed in triplicate according to the standard addition method. The slopes of the curves corresponding to aqueous standards and those corresponding to extracts spiked with inorganic mercury were statistically compared to check for possible matrix interference. Different sample treatments were also compared to obtain inorganic mercury and total mercury concentrations in good agreement with certified values in a minimum analysis time. Methylmercury could be determined by difference between total mercury and inorganic mercury, since methylmercury was the most common organomercury species present in the environmental samples.

The use of 10^{-4}% w/v sodium borohydride as reducing agent for inorganic mercury determination in the extracts obtained by sample treatment in alkaline medium was inadequate, because the inorganic mercury sensitivity decreased when successive replicates were analyzed. The effect of sodium borohydride concentration on sensitivity for inorganic mercury determination in the biological control sample was studied to solve this problem (Fig. 1). The slope of the standard addition curve increased with sodium borohydride concentration. However, a sodium borohydride concentration of 0.05% w/v was selected for further experiments, since greater sodium borohydride concentrations gave an absorbance signal for methylmercury.

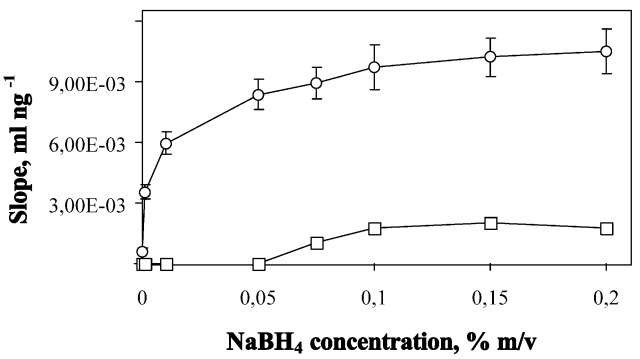

Fig. 1. Effect of the sodium borohydride concentration on the sensitivity of inorganic mercury determination in alkaline extracts of QC 91-LH1 spiked with methylmercury (□) and inorganic mercury (○).

The results obtained for inorganic mercury and total mercury determination in fish tissue and sediment samples by conventional and microwave-assisted extraction in alkaline or acid medium are shown in Table 4. It can be observed that the concentrations found for inorganic mercury and total mercury were only in good agreement with the certified values (t-test, $P=0.05$) for NRC DOLT-2 and NRC DORM-2 when 25% w/v tetramethylammonium hydroxide was used as extractant. However, the differences between both concentrations were significant (t-test, $P=0.05$) for inorganic mercury in QC 91-LH1 and total mercury in NIST 1944 and NIST 2704 using the same extractant. The inorganic mercury recovery was approximately 20% in QC 91-LH1 and total mercury recovery was approximately 7.7% in both sediment reference materials. The microwave energy had no influence on extraction efficiency in the alkaline medium. The low concentration recovered for inorganic mercury in QC 91-LH1 seems to be related to matrix effects involved in analysis of the extracts obtained from wet samples rather than low efficiency of the extraction procedure, since the percentage of inorganic mercury in this sample was 95% and the total mercury recovery was higher than 95%. As a consequence of the low concentration of total mercury recovered from sediment reference materials using the conventional extraction procedure in alkaline medium, it was not accelerated by microwave energy. Furthermore, matrix effects on the analytical response for extracts obtained using both alkaline digestion procedures were similar for each sample, as can be observed in Table 5, being usually more pronounced for inorganic mercury determination.

Table 4
Determination of inorganic mercury and total mercury in reference materials

Sample treatment	Mercury ($\mu g\ g^{-1}$)				
	NRC DOLT-2	NRC DORM-2	QC 91-LH1	NIST 1944	NIST 2704
Total mercury determination					
Certified	1.99±0.10	4.64±0.26	28.2±1.1	3.4±0.5	1.44±0.07
CE-ALK	2.16±0.36	4.51±0.44	28.5±3.0	0.259±0.572	0.111±0.262
MAE-ALK	2.11±0.27	4.70±0.59	26.8±3.0	–	–
CE-AC	1.93±0.29	4.74±0.42	27.7±3.3	2.11±0.34	1.10±0.27
MAE-AC	1.96±0.21	4.55±0.33	28.5±3.5	3.42±0.14	1.37±0.23
Inorganic mercury determination					
Certified[a]	1.30±0.11	0.170±0.412	26.8±1.1	–	–
CE-ALK	1.43±0.23	0.175±0.030	5.59±1.48	0.133±0.205	0.146±0.256
MAE-ALK	1.42±0.12	0.181±0.036	5.79±0.13	–	–
CE-AC	1.25±0.19	0.178±0.031	25.8±3.4	1.90±0.34	0.827±0.037
MAE-AC	1.37±0.15	0.166±0.028	25.9±2.7	3.28±0.25	1.29±0.31

CE, conventional extraction; MAE, microwave-assisted extraction; ALK, alkaline medium; AC, acid medium. Results are average value±confidence limit ($\bar{x}\pm ts/\sqrt{n}$) ($P=0.05$).

[a] The inorganic mercury concentrations were calculated as difference between certified total mercury and methylmercury concentrations.

Table 5
Slope values of the standard addition curves for the determination of inorganic mercury and total mercury in reference materials

Sample treatment	Slope (10^3 ml ng^{-1})				
	NRC DOLT-2	NRC DORM-2	QC 91-LH1	NIST 1944	NIST 2704
Total mercury determination					
CE-ALK	5.5±0.3	4.7±2.2	7.7±0.3	9.1±0.8	8.5±0
MAE-ALK	6.9±0.3	5.8±0.6	7.2±0.1	–	–
CE-AC	5.9±0.4	5.4±0.4	5.2±0.5	15±1	15±1
MAE-AC	9.0±0.6	9.9±0.5	9.5±0.3	15±1	16±1
Inorganic mercury determination					
CE-ALK	3.4±0	4.3±0.1	8.3±0.7	15±1	4.6±0.7
MAE-ALK	3.1±0.1	4.2±0.2	8.4±1.3	–	–
CE-AC	5.2±0.3	5.8±0.5	6.4±0.9	24±1	24±0
MAE-AC	12±1	15±2	14±2	24±1	23±1

CE, conventional extraction; MAE, microwave-assisted extraction; ALK, alkaline medium; AC, acid medium. Results are average value±standard deviation ($n=3$).

Acid extraction procedures were applied to the leaching of mercury species from biological and sediment samples. Inorganic mercury and total mercury concentrations found following conventional and microwave-assisted extraction were in good agreement with the certified values (*t*-test, $P=0.05$) for NRC DOLT-2, NRC DORM-2 and QC 91-LH1. However, the matrix interference on both mercury determinations diminished when acid microwave-assisted extraction was used for these samples. On the other hand, total mercury concentrations found in NIST 1944 and NIST 2704 were: (i) not in good agreement with certified values (*t*-test, $P=0.05$) using conventional extraction with 6 mol l^{-1} hydrochloric acid; and (ii) in good agreement with the certified values (*t*-test, $P=0.05$) using microwave-assisted extraction with the same extractant. Both sediment reference materials were not certified for inorganic mercury, but the results obtained for inorganic mercury were similar to those for total mercury. The analysis of sediment SRMs suggested that the mercury present in the material is inorganic mercury. It is in good agreement with the low methylmercury contents usually present in sediments. It is interesting to note that there was no matrix interference on the inorganic mercury and total mercury determination in the acid sediment extracts (Table 5), which permitted direct determination using the calibration method with aqueous standards instead of the standard addition method.

4. Conclusions

The proposed methodology permitted determination of inorganic and total mercury with sodium borohydride as the sole reducing agent, thus avoiding the use of oxidizing agents. The sample throughput was 28 samples/h. The detection limits were better than those previously published in relation to mercury speciation in FI-CV-AAS systems without chromatographic separation. Furthermore, the detection limit for inorganic mercury was lower than that for total mercury. Total mercury and inorganic mercury were quantitatively recovered from spiked water samples, which permitted verification of the method performance. Four different sample treatments were compared in terms of extraction efficiency of mercury species and matrix interferences on mercury determination in biological and sediment reference materials. The best recovery values were obtained with 25% w/v tetramethylammonium hydroxide for biological CRMs or 6 mol l^{-1} hydrochloric acid for all biological and sediment samples studied.

A microwave-assisted extraction method was selected for the preparation of all samples analyzed in this work because: (i) the recovery of mercury species was equal to or better than that obtained by the conventional extraction method, resulting in concentrations in good agreement with the certified values for each material; (ii) the least matrix interference was observed for analysis of

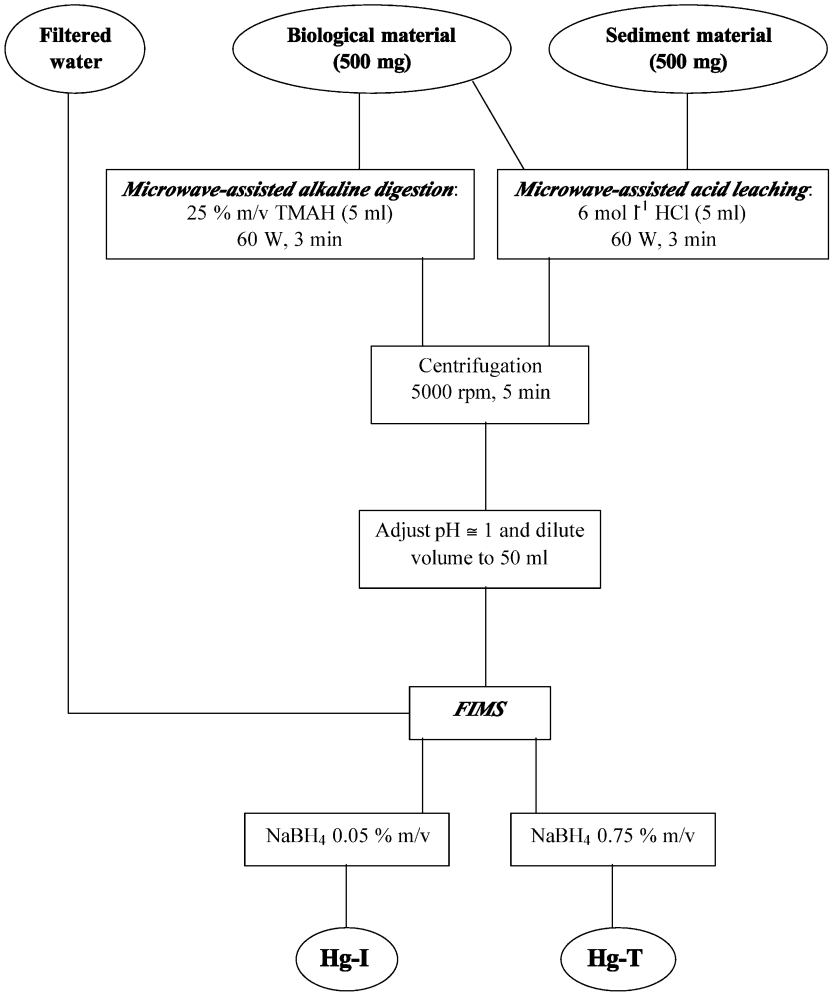

Fig. 2. Schematic diagram of the methodology proposed for mercury speciation in several environmental samples.

these extracts; and (iii) the time required for sample preparation was reduced from 3 h to 3 min compared to the conventional procedure. A schematic diagram of the proposed methodology for mercury speciation in several biological and environmental samples is shown in Fig. 2.

Acknowledgments

Two grants supplied by Xunta de Galicia and Universidad de Vigo are gratefully acknowledged by S. Río Segade.

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