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Evaluation of various sample pre-treatment methods for total and inorganic mercury determination in biological certified reference materials by CVAAS technique

Research Article

Henryk Matusiewicz*, Ewa Stanisz

Politechnika Poznańska, Department of Analytical Chemistry,
60-965 Poznań, Poland

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Abstract: Sample preparation methods for non-separation cold vapor atomic absorption spectrometry (CVAAS) sequential inorganic mercury speciation in biological certified reference materials (CRMs) were investigated. The methylmercury concentration was calculated as the difference between total and inorganic mercury. Microwave-assisted decomposition method, and three ultrasonic extraction procedures based on acid leaching with HCl and HCOOH and solubilization with TMAH were employed as sample preparation methods. The replacement of a sample decomposition procedure by extraction prior to analysis by CVAAS, as well as the aspect of speciation analysis is discussed. The limits of detection in the sample were determined as 50 and 10 ng L^{-1} for inorganic and total mercury, which corresponds to absolute detection limits of 40 and 8 ng g^{-1} for inorganic and total mercury, respectively. The results were in good agreement with the 95% confidence level t-test of the certified values for total and inorganic mercury in the reference materials investigated. From the analysis of the CRMs, it was evident that the difference between the total and inorganic mercury concentrations agrees with the methylmercury concentration. The relative standard deviation was better than 11% for most of the samples.

Keywords: *Hydrolyzed polyacrylonitrile fibres • Mercury speciation • Sample pre-treatment • Cold vapor atomic absorption spectrometry • Certified reference materials*

1. Introduction

Mercury (Hg) is one of the most hazardous pollutants in the environment. It is exists in the environment in inorganic (elemental mercury (Hg^0) and (Hg^{2+})) and organomercury forms. The study of methylmercury (CH_3Hg^+) is particularly important due to its high toxicity and abundance among organomercury species in the environment. As a result, more sensitive, accurate and rapid analytical techniques are required to monitor Hg species in different environmental and biological samples [1,2].

The determination of Hg in environmental and biological samples has led to significant progress in the development of analytical measurement techniques. The most popular approach is still the cold vapor generation (CV). This technique consists on the mineralization of mercury species to Hg^{2+} through acidic attack and the reduction to the element reaction with $SnCl_2$ or $NaBH_4$ [3]. On the other hand, a variety of detection techniques combine a separation technique with element-specific detection, such as long-path quartz tube atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS) and *in situ* collection of the cold vapor coupled to an electrothermal atomizer for AAS. The information of organic and inorganic forms of mercury could also be obtained with this same instrumentation using different reducing agents with different reducing powers. Currently, the complexity and high cost of this instrumentation have led to the use of a number of non-separation speciation schemes for mercury speciation employing CVAAS

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* *E-mail: Henryk.Matusiewicz@put.poznan.pl*

[4-6]. A common indirect speciation approach is the subtraction of inorganic mercury from its total fraction. Without any prior decomposition procedure, inorganic mercury is selectively determined, otherwise total mercury is determined.

The determination of total mercury and its inorganic speciation in biological samples requires careful consideration of sample pretreatment. A substantial number of different methods are currently available for this procedure [2]. There are several approaches that describe the preparation of biological samples prior to the determination of mercury and inorganic mercury species. For example, simple and inexpensive procedures like the use of tetramethylammonium hydroxide (TMAH) and formic acid for sample solubilization have been used with the main atomic spectrometric techniques, including cold vapor atomic fluorescence or absorbance spectrometry (AFS or AAS). However, there is still a deficit of faster and simpler procedure to distinguish between inorganic and other forms of mercury. For speciation of mercury in biological samples, a fundamental step is the sample preparation procedure before CVAAS analysis. Usually, microwave-assisted sample digestion has been recommended. Nevertheless, due to the increasing demand for sample trace element analysis, and the increasing concern for occupational and environmental exposure to mercury, sample preparation procedures with minimal handling and time consumption are highly desirable for environmental laboratories routine analysis.

Therefore, the goal of this work is to develop a simple and reliable procedure that reduced the sample preparation time required for the determination of total and inorganic mercury as well as methylmercury, in biological samples. The organic mercury will be calculated as the difference between the total mercury and the inorganic mercury. For that purpose, the use of an inexpensive, sensitive and reliable speciation ultrasound extraction methods using CVAAS technique are proposed. A microwave-assisted digestion was also applied for sample pre-treatment for comparative purposes.

2. Experimental Procedure

2.1. Instruments and apparatus

The analyses were carried out with a mercury analyser (Model Aula-254, Mercury Instruments, GmbH, Karlsfeld, Germany) for Hg measurement. A mercury electrodeless low pressure discharge lamp was used as the radiation source. Peak absorbance of the transient signal was chosen for quantification. Instrumental parameters were set up as follows: lamp intensity, 4-20 mA; wavelength, 253.7 nm; spectral bandpass, 0.4 nm; quartz cell temperature, 50°C; measurement mode, peak height. Deuterium lamp background correction was used during the whole analysis. A thermoelectric gas dehumidifier and heating of the optical cell eliminated the moisture and prevented interferences from water vapor. A general view of the CVAAS system applied in this experiment is shown in Fig. 1.

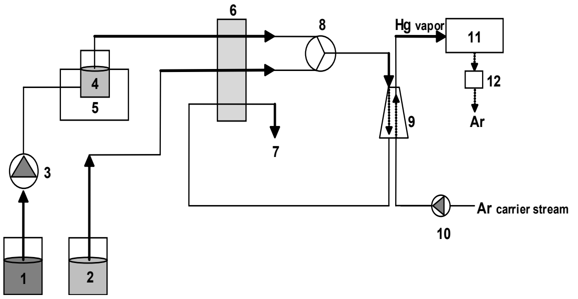

Figure 1. Schematic diagram of CVAAS system: 1-washing solution, 2-reducing agent ($SnCl_2$), 3-rinse pump, 4-sample solution, 5-autosampler, 6-peristaltic pump, 7-waste, 8-injection valve, 9-cross-flow reactor, 10-mass-flow controller, 11-AULA atomic absorption spectrometer, 12-atomic absorption cell.

A UniClever microwave sample preparation system (Plazmatronika, Wrocław, Poland) equipped with high-pressure TFM-PTFE vessel was used for sample digestion. The vessel capacity was 110 mL and the maximum pressure and maximum temperature were 100 bar (1 bar = 10^5 Pa) and 300°C, respectively.

A Sonopuls HD 70 ultrasonic cell disruptor/homogenizer (70 W, 20 kHz, Bandelin, Germany) equipped with a 3-mm titanium microtip was used for Hg extraction processes. Ultrasonic energy irradiation was fixed at any desired level using a power setting in the 10-65 W for 3-mm titanium microtip. Additionally, a centrifuge (Model EBA 20, Hettich, Germany) was employed for phase separation after extraction procedures.

2.2. Reagents, chemicals and gases

Compressed argon gas of N-50 purity (99.999%) obtained from BOC GAZY (Poznań, Poland) was employed as the carrier gas without further purification.

Standard solutions of inorganic mercury - Hg^{2+} (as $HgCl_2$) were prepared from a 1000 mg mL^{-1} Hg atomic absorption standard (Titrisol grade, Merck, Darmstadt, Germany) stabilizing with potassium dichromate (5% m/v, GR, Merck). The stock solutions were stored at 4°C prior to use. All working standard solutions of Hg were prepared daily to prevent any possible species change, due to dilution of the appropriate stock solution aliquots with high-purity water.

Stannous chloride (10% m/v for Hg^{2+} and 2% m/v for total Hg determination), used as a reducing agent, was prepared by dissolving the appropriate mass of stannous chloride dihydrate (Merck) in concentrated hydrochloric acid (32% v/v extra pure, Merck) and by diluting to the desirable volume with ultra-pure water. The hydrochloric acid concentration in the reducing agent was 2 mol L^{-1}. Additionally, a rinsing solution NH_4OCl (0.1% (m/v)) was used.

A commercial solution of 25% m/v tetramethylammonium hydroxide (TMAH) (Fluka, Buchs, Switzerland) in water was used to treat samples for alkaline solubilization.

Formic acid (89-91%, GR, Merck) was used to solubilize the biological tissues.

All mineral acids (69% HNO_3, 32% HCl) and hydrogen peroxide 30% (v/v) of the highest quality (Suprapur, Merck) were used. High-purity water: deionized water (model DEMIWA 5 ROSA, Watek, Czech Republic), and doubly distilled water (quartz apparatus, Bi18, Heraeus, Hanau, Germany) were also used throughout the experiments.

2.3. Certified reference materials

Validation of the methods described in this work was performed using three certified reference materials: DORM-2 (Dogfish Liver), DOLT-2 (Dogfish Liver) and TORT-2 (Lobster Hepatopancreas) from the National Research Council of Canada, Ottawa, Canada. The certified reference values are available for mercury, inorganic mercury and methylmercury for assessment of the method accuracy. All solid reference materials were used as bottled, without further grinding and sieving.

2.4. Microwave-assisted digestion for total Hg determination

To evaluate the efficiency of the applied extraction procedures, the amount of total mercury, present in certified reference materials, were determined by CVAAS after decomposition with closed microwave-assisted acid digestion, which should ensure complete dissolution of the samples.

Approximately 250 mg of powdered organic reference material was placed in the TFM-PTFE vessel of the microwave digestion system and moistened by 1 mL of 30% H_2O_2. Then, 4 mL of concentrated HNO_3 was added. The sample was heated for 20 min at 300 W. After mineralization, the clear digested solution was transferred into 20 mL calibrated flask and diluted to volume with water. Before further analysis this solution was appropriately diluted depending on the concentration level of the element. In all cases, a corresponding blank was also prepared according to the above microwave-assisted digestion procedure.

2.5. Ultrasonic extraction procedures

For mercury species, three ultrasonic extraction procedures, adapted from literature [5,7-9], were evaluated. Three extraction solvents: HCl, TMAH and HCOOH were applied. All sonications were performed in continuous (non-pulsed) mode with a 3-mm diameter titanium microtip immersed into the sample solution. A procedural blank was prepared along with the samples for quality assurance purposes.

2.5.1. Ultrasonic HCl extraction

250 mg of sample and 5 mL of 5 mol L^{-1} hydrochloric acid were placed in centrifuge tube and were sonicated at fixed ultrasound amplitude of 35 W for 5 min. After extraction, the sample solution was centrifuged at 1600 rpm for 15 min. The supernatant was quantitatively transferred into a 10 mL calibrated quartz flask and diluted to the desirable volume with water. Before further analysis this was appropriately diluted depending on the concentration level of the mercury.

2.5.2. Ultrasonic alkaline TMAH extraction

Nominal 250 mg sub-samples of reference material were weighed into 30 mL pre-cleaned polypropylene screw-cupped cups with additional 5 mL of 25% m/v TMAH. Once the 5 min reaction of the sample with the TMAH was completed, 15 mL of water was added. Suspensions were pretreated by sonication at 50 W ultrasonic probe power for 5 min. The final concentration of TMAH in sample solution was 6.25% (m/v). Before further analysis this was appropriately diluted depending on the concentration level of the mercury.

2.5.3. Ultrasonic HCOOH extraction

Nominal 250 mg sub-samples of reference material were weighed into 30 mL pre-cleaned polypropylene screw-capped cups and additional 20 mL of ca. 90% m/v formic acid. Once the 5 min the reaction of the sample with the HCOOH was completed, suspensions were pretreated by sonication at 50 W ultrasonic probe power for 5 min. Before further analysis this solution was appropriately diluted depending on the concentration level of the mercury.

2.6. Mercury cold vapor generation

2.6.1. Total mercury determination

To determine the total mercury content, the dried samples (ca. 250 mg) were digested with 4 mL of 69% HNO_3 and 1 mL of 30% H_2O_2 in a microwave digestion system following the procedure indicated in section 3.1. Total mercury determination, on the digested solution, was performed by the reduction of all organic and inorganic Hg species to volatile Hg^0 with $SnCl_2$ and detected by AAS. During the analysis, a volume ranging from 0.5 to 2.0 mL of the sample solution, containing the digested reference material, was made up to volume in a 10 mL calibrated flask with water. 10 mL of this solution was placed in the reaction flask for vapor generation. Mercury vapor was generated in 2 mol L^{-1} HCl medium using 2% (w/v) $SnCl_2$ reducing agent. The Hg vapor was transferred to the quartz cell by an Ar stream (70 mL min^{-1}). The total Hg concentration of the samples was determined by standard addition technique using CVAAS.

2.6.2. Inorganic mercury determination

The inorganic mercury was determined after ultrasonic HCl, TMAH solubilization, and HCOOH extractions following the procedures indicated in sections 3.2.1., 3.2.2. and 3.2.3. For inorganic mercury determination, 0.5 to 3.0 mL of the sample solution containing the supernatant of solubilized tissue, with an additional amount of conc. HCl, was made up to the desirable volume in a 10 mL calibrated flask with water. The HCl concentration in the sample solution was 5 mol L^{-1}. 10 mL of this solution was placed in the reaction flask for vapor generation. Mercury vapor was generated in 2 mol L^{-1} HCl medium using 10% (w/v) $SnCl_2$ reducing agent. The Hg vapor was transferred to the quartz cell by an Ar stream (70 mL min^{-1}). The total Hg concentration of the samples was determined by standard addition technique by CVAAS.

2.6.3. Indirect determination of methylmercury

Calculation: the methylmercury concentration (CH_3Hg^+) was calculated as the difference between total (Hg) and inorganic mercury (Hg^{2+}) from the equation:

$$Hg_{total} - Hg_{inorganic} = Hg_{organic}$$
$$(Hg - Hg^{2+} = CH_3Hg^+)$$

where Hg_{total} and $Hg_{inorganic}$ are the quantities of mercury.

3. Results and Discussion

3.1. Total mercury

Nitric acid and hydrogen peroxide have been proposed as sample decomposition reagents for the microwave-assisted digestion procedure. For a single-stage power and time setting, different volumes of HNO_3 and H_2O_2, and different times and power settings of the focused microwave Teflon closed bomb system, were tested to ensure total recovery of Hg after sample treatment. The addition of 1 mL of H_2O_2 with 4 mL of HNO_3 improves the efficiency of the digestion, providing a clearer solution and greater accuracy in the analysis of reference materials (samples were irradiated at a 300 W power setting for 20 min).

The analytical determination of total Hg based on CVAAS requires the optimization of several operated parameters. Mercury reduction was carried out with an acidic solution (HCl) of $SnCl_2$ as is extensively recommended in the literature. The reducing agent concentration of 2% $SnCl_2$ was used as well as hydrochloric acid concentration of 2 mol L^{-1} .

Table 1 lists the results of total Hg content for each reference material obtained by CVAAS tests. The obtained results gave a range of total Hg between 0.28 to 4.71 μg g^{-1}. The total mercury content with regard to the type of the reference materials tested was widely variable. This was expected, since the investigated materials were chosen to test samples with different content of mercury. A good agreement with certified values was obtained.

Table 1. Analytical results for the determination of total mercury in certified reference materials by CVAAS.

Material	Total Hg (μg g^{-1}) Found	Certified
DORM-2	4.71 ± 0.31	4.64 ± 0.26
DOLT-2	1.98 ± 0.12	1.99 ± 0.10
TORT-2	0.28 ± 0.03	0.27 ± 0.06

The results were obtained by standard addition technique
The values are means ± standard deviation (n=5)

3.2. Optimization of ultrasound extraction conditions

Assuming that all the CRMs would have behaved similarly during the ultrasonic extraction, DOLT-2 was chosen as the trial sample. This material was used to optimize the experimental parameters to produce 100% relative extraction efficiency. Evaluated parameters were time of sonication, ultrasonic power, and volume of extractants (Figs. 2, 3 and 4). The sample mass was

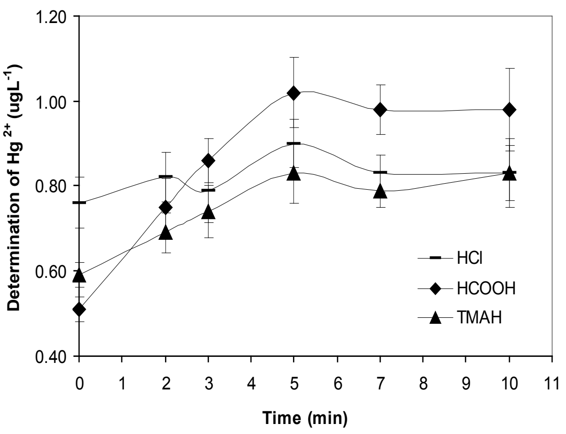

Figure 2. Effect of ultrasonication time on CVAAS response from 1 µg L^{-1} solutions of Hg^{2+} from DOLT-2 (HCl, 5 mol L^{-1}; HCOOH, *ca.* 90%; TMAH, *ca.* 6%; ultrasonic power, 40 W).

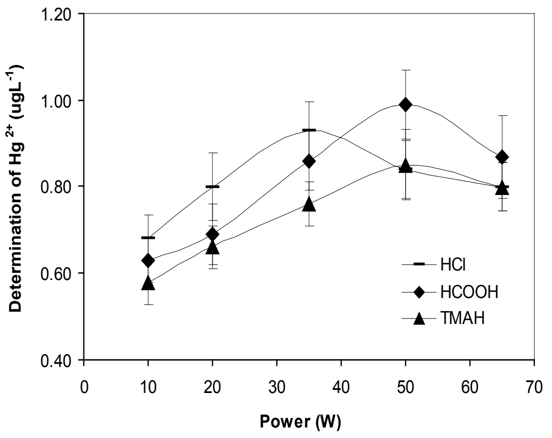

Figure 3. Effect of ultrasonic power on CVAAS response from 1 µg L^{-1} solutions of Hg^{2+} from DOLT-2 (HCl, 5 mol L^{-1}; HCOOH, *ca.* 90%; TMAH, *ca.* 6%; ultrasonication time, 5 min).

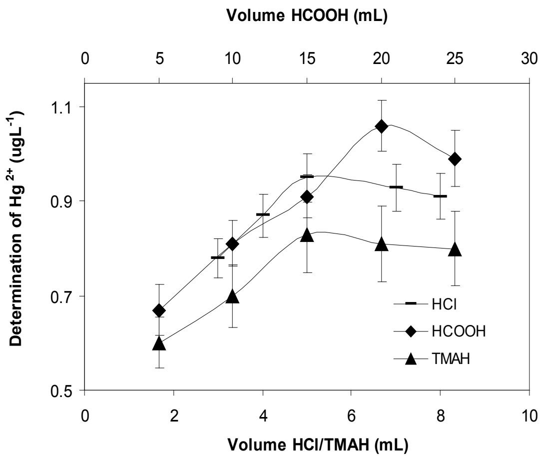

Figure 4. Effect of volume of extractant on CVAAS response from 1 µg L^{-1} solutions of Hg^{2+} from DOLT-2 (HCl, 5 mol L^{-1}; HCOOH, *ca.* 90%; TMAH, *ca.* 6%; ultrasonication time, 5 min; ultrasonic power, 40 W).

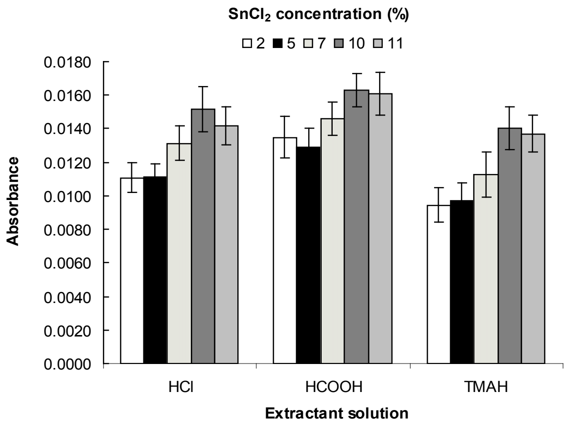

Figure 5. Effect of concentration of $SnCl_2$ on CVAAS response from 1 µg L^{-1} solutions of Hg^{2+} from DOLT-2 (HCl, 5 mol L^{-1}; HCOOH, *ca.* 90%; TMAH, *ca.* 6%; ultrasonication time, 5 min; ultrasonic power, 40 W).

kept constant for all experiments.

The importance of this study was to evaluate the extraction efficiency of three selected extractant used for Hg_{total}, $Hg_{inorganic}$ and $Hg_{organic}$ determination in biological samples. It was observed that the type of extraction solution was a significant factor that affects the results for mercury species. The extraction efficiencies in the ultrasound procedure tested are generally unknown because transformation of species is not assessed by neither standard extraction nor sample preparation protocols and analyses. The performance of these extraction procedures was evaluated by analysis of CRMs with certified values for total mercury and CH_3Hg^+, respectively.

Since the separated determination of inorganic mercury and total mercury with the use of different concentrations of sodium tetrahydroborate ($NaBH_4$) was not possible to carry out with the proposed system, it was further evaluated the use of a different reducing agent, such as $SnCl_2$. Then, optimization of the $SnCl_2$ concentration was performed as a compromise between mercury sensitivity and specificity for inorganic Hg determination in DOLT-2 (Fig. 5).

The optimized conditions for the ultrasonic extraction procedure are summarized in Table 2.

Table 2. Optimized ultrasound extraction conditions.

Extractant	Optimized parameters			
	$SnCl_2$ concentration (%)	Ultrasound power (W)	Sonication time (min)	Extractant volume (mL)
5 mol L^{-1} HCl	10	35	5	5
ca. 90% HCOOH	10	50	5	20
ca. 6% TMAH	10	50	5	5

DOLT-2 sample weight, 250 mg

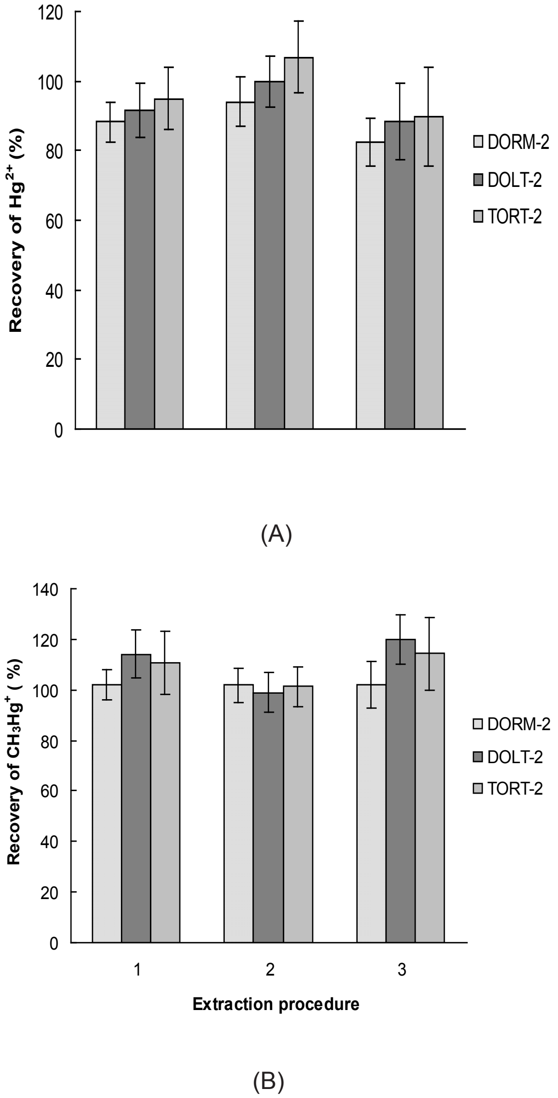

Figure 6. Effect of the ultrasound extraction effectiveness on: (A) inorganic mercury (Hg^{2+}) recovery and (B) methylmercury (CH_3Hg^+) recovery when different extraction solvents are used: 1- HCl, 2- HCOOH, 3- TMAH. The samples were sonicated for 5 min at room temperature with power 35 W for HCl and 50 W for HCOOH and TMAH. The uncertainty represents the standard deviation of three replicates.

3.3. Comparison of effect of extractant for ultrasound extraction procedures

Three extraction media were tested to investigate their efficiency on the extraction of mercury from biological samples. Hydrochloric acid (HCl), tetramethylammonium hydroxide (TMAH) and formic acid (HCOOH) were used as extractants. The listed chemicals have been commonly used in speciation analysis of mercury in biological samples.

A comparison of the analytical results obtained with the three different extraction procedures for the mercury is given in Fig. 6.

A reasonable question is whether there is an optimum extraction procedure. However, there are still uncertainties to answer this question. Our general observations on the comparison of three extraction procedures are as follows. As can been seen (Figs. 2, 3 and 4), the efficiency of the extraction strongly depends on the extractant solution. Generally, TMAH extracted the least amount of inorganic mercury (Hg^{2+}), but the efficiency of formic acid to extractant Hg^{2+} and CH_3Hg^+ was close to 100%. Thus, for Hg^{2+} and CH_3Hg^+ extraction with formic acid can replace the conventional decomposition procedure by concentrated acid(s).

3.4. Analytical characteristics

The severe memory effect of the instrumentation is a significant and difficult problem to overcome during the mercury determination by cold vapor AAS. This problem has been attributed to a combination of several factors [10]. The consequences of these effects include non-linear calibration graphs, long washout times, decreasing sensitivity with time, and signal dependence on the matrix.

In this study, potential mercury memory effect was observed. To overcome this limitation, it was necessary to use a solution containing NH_4OCl (0.1% m/v) and

Table 3. Figures of merit for the determination of total mercury (Hg) and inorganic mercury (Hg^{2+}) in solutions by CVAAS.

Parameter	System			
	Total Hg	$Hg^{2+\,a}$	$Hg^{2+\,b}$	$Hg^{2+\,c}$
Limit of detection (LOD) (ng L^{-1})	10	30	30	50
Limit of detection (LOD) (ng g^{-1})	8	12	24	40
Limit of quantification (LOQ) (ng L^{-1})	33	100	100	167
Limit of quantification (LOQ) (ng g^{-1})	27	40	80	133
Blank (ng L^{-1})	70	80	80	80
Blank (ng g^{-1})	56	32	64	64
Relative standard deviation (RSD)[d] (%)	7	8	8	11

[a] 5 mol L^{-1} HCl extraction
[b] ca. 90% HCOOH extraction
[c] ca. 6% TMAH solubilization
[d] n=5

Table 4. Validation of methods for total, inorganic and methylmercury in selected ertified reference biological materials by CVAAS using the optimized ultrasonic extraction procedures. Obtained values (average value ± standard deviation) in µg g^{-1} (n=5).

Reference material code	Extraction reagent/ technique	Determined			Certified		
		Total Hg [a]	Hg^{2+}	CH_3Hg^+ [b]	Total Hg [a]	Hg^{2+}	CH_3Hg^+ [b]
DORM-2	MW digestion	4.71 ± 0.31			4.64 ± 0.26		
	5 mol L^{-1} HCl		0.15 ± 0.01	4.56 ± 0.27		0.17 ± 0.41	4.47 ± 0.32
	ca. 90% HCOOH		0.16 ± 0.01	4.55 ± 0.31			
	ca. 6% TMAH		0.14 ± 0.01	4.57 ± 0.42			
DOLT-2	MW digestion	1.98 ± 0.12			1.99 ± 0.10		
	5 mol L^{-1} HCl		1.189 ± 0.093	0.791 ± 0.076		1.297 ± 0.113	0.693 ± 0.053
	ca. 90% HCOOH		1.294 ± 0.095	0.686 ± 0.055			
	ca. 6% TMAH		1.149 ± 0.126	0.831 ± 0.083			
TORT-2	MW digestion	0.28 ± 0.03			0.27 ± 0.06		
	5 mol L^{-1} HCl		0.112 ± 0.010	0.168 ± 0.021		0.118 ± 0.061	0.152 ± 0.013
	ca. 90% HCOOH		0.126 ± 0.013	0.154 ± 0.012			
	ca. 6% TMAH		0.106 ± 0.015	0.174 ± 0.025			

[a] Total Hg = Hg^{2+} + CH_3Hg^+
[b] Calculated as difference between total Hg and inorganic Hg values

employ 1 minute washing time. Then, after each running, the solution was passed through the system for one minute. Table 3 gives the typical residual blank contributions which resulted after rinsing procedure.

All figures of merit of the proposed systems and the methods for inorganic and total mercury determination are shown in Table 3. The limits of detection (LOD, 3 s) and quantification (LOQ, 10 s) from the blanks were calculated from the standard deviation of 10 consecutive measurements. Generally, the extraction procedure chosen had little influence on the detection limit of mercury analysis. The best limit of detection was achieved for total Hg determination - 10 ng L^{-1}. For ultrasonic HCl and HCOOH extractions, limits of detection were 30 ng L^{-1}.

The precision, evaluated as the average relative standard deviation (RSD%), was better than 11%.

3.5. Determination of inorganic mercury in certified reference materials

In most of the CRMs, either inorganic or methylmercury are found to be at much higher concentrations relative to the other species, depending on the nature of the material. In all cases, the difference between total and inorganic mercury was taken as methylmercury, leading to a good agreement with the certified reference values.

The results obtained for the determination of total Hg, Hg^{2+} and CH_3Hg^+ in several CRM are summarized in Table 4. The determined values for inorganic mercury agree with the certified total mercury, inorganic mercury and methylmercury contents within the 95% probability level of the certificate to the reference materials investigated. Despite the uncertainty associated to an indirect procedure, the organic mercury concentration, calculated as the difference between the values obtained with and without sample extraction using HCOOH (DOLT-2), also agrees with the certified methylmercury concentration. This demonstrates that most of the organic mercury obtained by arithmetical difference is probably methylmercury. Exceptions are DORM-2 and TORT-2, for which the inorganic Hg concentrations were found to be slightly higher. The accuracy was verified by comparing with a total microwave-assisted acid digestion (Table 4).

4. Conclusions

The present study is a critical comparison of different reagents used with the ultrasound extraction method for bringing mercury to a solution. The results obtained using three different extraction reagents indicate that no single method appears to be ideal for the accurate CVAAS determination of total and inorganic mercury in the certified reference materials. However, a simple ultrasound-assisted extraction method for the determination of Hg^{2+} and total Hg (Hg^{2+} plus CH_3Hg^+), in biological certified reference materials treated with HCOOH or digested in a microwave system. The determination of total mercury at room temperature, after CRM acid digestion in closed microwave system, assures a limit of detection similar to the one obtained for Hg^{2+}. In addition, the method allows estimating the concentration of methylmercury without handling a methylmercury standard. The procedure for the proposed speciation analysis does not require any chromatographic techniques, requiring only a AAS instrument with the simple cold vapor generation device. Simplicity and high efficiency without using chromatographic techniques are some of the qualities of the proposed method, being adequate for speciation analysis of mercury in biological samples. The main features of the present method are the easy implementation and sample preparation since no matrix separation is necessary. The limit of detection for total Hg determination was 10 ng L^{-1}. The limitation of the method using HCOOH for ultrasonic extraction is relatively higher than the limit of detection: 30 ng L^{-1}. This drawback could be overcome by coupling the unit to more powerful detectors, such as AFS or ICP-MS, or by pre-concentrating Hg vapor after the derivatization step.

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