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Slurry sampling in serum blood for mercury determination by CV-AFS

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ABSTRACT

The heavy metal mercury (Hg) is a neurotoxin known to have a serious health impact even at relatively low concentrations. A slurry method was developed for the sensitive and precise determination of mercury in human serum blood samples by cold vapor generation coupled to atomic fluorescence spectrometry (CV-AFS). All variables related to the slurry formation were studied. The optimal hydrochloric concentration and tin(II) chloride concentration for CV generation were evaluated. Calibration within the range 0.1-10 $\mu g\ L^{-1}$ Hg was performed with the standard addition method, and compared with an external calibration. Additionally, the reliability of the results obtained was evaluated by analyzing mercury in the same samples, but submitted to microwave-assisted digestion method. The limit of detection was calculated as 25 $ng\ L^{-1}$ and the relative standard deviation was 3.9 % at levels around of 0.4 $\mu g\ L^{-1}$ Hg.

Keywords: Slurry sampling; Human serum blood; Mercury; Atomic fluorescence spectrometry

Introduction

Mercury is a high toxic element because of its accumulative and persistent nature in the environment and biota [1]. As a result, increasingly sensitive, accurate and rapid analytical techniques are required to monitor Hg in biological and environmental samples [2].

Atomic fluorescence spectrometry (AFS) detection, especially coupled with the cold vapor (CV) technique, is becoming popular and replacing atomic absorption spectroscopy (AAS) for mercury analysis due to its simple instrumentation and ultra low detection limits, which can be evidenced by its approval by the US Environmental Protection Agency [2]. Morita et al. [3] reviewed AFS methods for determining mercury. Furthermore, the principles of AFS of Hg and applications of CV-AFS have been discussed [4, 5].

Measurements of Hg in blood are performed in order to determine whether adverse health effects are likely to occur in human beings [6]. Normal concentrations of total whole blood Hg are typically less than 5.0 μg L^{-1} in adults [7]. However more recent data in the non-institutionalized US population suggest that the 95% for whole blood Hg is 2.30 μg L^{-1} for young children and 7.10 μg L^{-1} for adult females [8].

A critical step during Hg determination in biological samples is the conversion of organomercury species into inorganic Hg ions, which are then reduced to elemental Hg. In the specific case of blood matrices, acid digestion followed by cold vapor generation has been proposed as a valuable alternative. However these methodologies require a heating step which may affect the accuracy of the analysis due to possible mercury loses in open systems, or contamination risks due to high sample manipulation.

Preliminary studies in this field [9-11] were focused on the determination of mercury in previously mineralized biological samples by CV-AFS. The use of dry-ashing,

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digestion in block or microwave-assisted acid digestion is a time-consuming process and requires excessively sample treatment which may affect the accuracy of Hg determinations. In this context, the slurry sampling appears as a good alternative for sample pretreatment. Slurries are solid dispersions in a liquid phase which can be transported as solutions, enabling the direct determination of mercury by ETAAS [12] or cold vapour generation coupled to ICP OES, AAS or AFS [5, 13-17]. This technique reduce the time required for analysis and risks of contamination by circumventing sample decomposition with wet or dry oxidation methods, minimizing mercury losses due to volatilization. Furthermore, slurry allows a sample pre-treatment simplification and detection limits improvement, applied to both organic and inorganic samples [18-23].

In this work, the elaboration of a procedure for rapid determination of mercury in blood serum using slurry sampling is discussed. For this purpose, all experimental variables related with the slurry preparation and mercury cold vapour generation were studied. Finally, the proposed methodology was applied to the determination of ultra-trace mercury levels in serum blood obtained from voluntary persons.

2. Experimental

2.1. Instrumentation

The measurements of mercury fluorescence were carried out with an Atomic Fluorescence Spectometer, AI 3300, Aurora Instruments (Vancouver, British Columbia Canada). The apparatus was equipped with a two-channel peristaltic pump for the continuous fluorescence measurements. Hollow cathode lamp for Hg from Aurora Instruments (Vancouver, British Columbia Canada) was employed as Hg fluorescence excitation source.

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The microwave digestion was performed with a domestic microwave oven (Philco, Ushuaia, Argentina) equipped with a magnetron of 2450 MHz and Milestone hermetically sealed, 1 cm wall thickness polytetrafluoroethylene (PTFE) reactors (100 mL internal volume). The digestion was carried out at maximum power of 700 W.

An ultrasonic bath, (Astrason Ultrasonic Clear, Farmingdale, N.Y. USA), was employed for slurries preparation.

2.2. Reagents

All reagents were of analytical-reagent grade and the presence of the mercury was not detected within the working range. A stock mercury standard (1000 µg mL^{-1}) was prepared from mercury (II) chloride, Merck (Darmstadt, Germany), in nitric acid, Merck, and raise to 1000 mL with ultrapure water.

$SnCl_2·2H_2O$ from Sigma (St. Louis, MO, USA) in 10% (v/v) HCl (both from Merck) was used as reductant agent. It was prepared by dissolving the salt in concentrated HCl, heating for 10 min and diluting with water.

Aqua regia was prepared by mixing HNO_3, from Fluka Sigma-Aldrich, (Steinheim, Germany) and HCl 1:3 (v/v) from the concentrated suprapure solutions. Antifoam 204, Sigma, was also used.

Ultrapure water (18.1 MΩ cm^{-1}) was obtained from Barnstead EASY pure RF water system (Iowa, USA).

2.3. Sample collection

Blood samples from 30 healthy adults from San Luis City were drawn from an antecubital vein using vacutainer tubes. Blood was transferred into a polystyrene tube, allowed to clot for 30 min. Then, the blood serum was prepared by three centrifugations of blood samples at $1000×g$ for 10 min in the same tube. The serum was then separated,

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transferred to a polystyrene tube and stored at −20 °C until analyzed. Hemolyzed samples were discarded.

2.4. Experimental procedure

The studies were focused on the appropriate conditions to obtain the best analytical signals as peak heights. The effect of the cold vapor generation conditions was studied, including the injection of a discrete sample volume. Special attention was paid to the acid treatment of slurries of serum blood in order to achieve the best analytical signal for Hg. The analytical features of the methodology were established from calibration data and from the analysis of real samples. Finally, data obtained from slurries samples were compared with those found after matrix removal.

2.5. Slurry sampling

This procedure started with the addition of aqua regia to 1.0 mL of serum. After that, a desired ammount of antifoam was added, and the mixtures were sonicated at room temperature for 10 min in order to obtain homogeneous dispersions. The analyte was determined experimentally as it is shown in table 1.

2.6. Microwave-assisted digestion procedure

Serum blood was introduced into the PTFE reactors. Then, 1.0 mL of concentrated nitric acid and 1 mL of hydrogen peroxide were added and the reactors were closed. Serum blood samples were digested in several steps applying different microwave power, i.e. MW power was held at 350 watts (3 min.), 350 watts (5 min.), 550 watts (5 min.), 550 watts (5 min.) The vessels were then removed from the oven and cooled at 20 °C, after that, they were vented and opened. At the end of this process, completely clear solutions were

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obtained. Digested samples were analyzed by CV-AFS following the procedure indicated before for slurries.

2.7. Experimental settings

Figure 1 shows the schematic diagram of the experimental setup. This is a flow injection manifold which includes two peristaltic pumps (P_1 and P_2), Tygon tubing and a two way-six port rotary valve with a PTFE sample loop.

Slurry loading step: with valve **V** in position **a** and pump **P_1** on, the slurry was loaded on the sample loop while the hydrochloric acid was flowing by the other line directly to the gas-liquid separator. At that stage, the base line (blank) was read.

Slurry injection step: once the background signal was established, valve **V** was switched to **b** position. At that time, the HCl solution passed through the sample loop carrying the slurry directly to the gas-liquid separator. The mercury present in the slurry reacts with the $SnCl_2$ and its fluorescence was induced by the radiation source. The final signal was recorded, and its height was proportional to the mercury concentration in the slurry.

The operation conditions were established and the determinations were carried out.

3. Results and discussions

In order to achieve the best analytical performance, several experimental variables were studied. These factors may be divided into two groups, i.e. critical factors pertaining to slurry preparation and factors pertaining to slurry analysis by CV-AFS.

3.1. Main variables of slurry preparation

The possible critical factors of slurry preparation occur during slurry preparation and during the introduction into the gas-liquid separator. These may include the lack of

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representativeness due to aliquot dosage and precision deterioration due to the difficulty in obtaining reproducible sampling.

3.1.1 Effect of aqua regia composition and volume

A number of liquid media have been used for slurry preparation. They are important not only as suspension media but also as analyte extracting media, contributing to the precision and accuracy of the method. Aqua regia is usually employed to prepare slurry of protein-rich matrixes [13, 15]. The formation of serum slurries was studied, monitoring the Hg fluorescence while the HCl-HNO$_3$ ratio was varied. The results obtained indicated that with a HCl:HNO$_3$ ratio of 3:1, the results were satisfactory. Additionally, the volume of aqua regia added was tested. We could verify that best results were obtained when the volume of aqua regia increased up to 1 mL. An excessive increase in the volume of aqua regia was avoided due to the obvious decrease in the sensitivity. Once aqua regia was added, ultrasonic was applied in order to form the slurry. This step was carried out at room temperature in order to avoid any mercury loses.

3.1.2. Effect of antifoam

The addition of a dispersant increases slurry stabilization and prevents foam formation and molecular agglomeration. We evaluated two different reagents in order to obtain slurries with long term stability, i.e. Antifoam A and Antifoam 204. Best results were observed when Antifoam 204 was added. Additionally, the effect of the antifoam concentration in the slurry was also studied. An antifoam concentration of 10 % v/v provided an appropriate dispersant effect to slurries, improving the mercury signal significantly.

3.2. Factors pertaining to slurry analysis by CV-AFS

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3.2.1 Effect of stannous chloride and hydrochloric acid

In order to maximize the mercury fluorescence signal, the variables affecting the cold vapor generation (i.e. $SnCl_2$ and HCl concentration) were optimized. We found that the minimum $SnCl_2$ concentration necessary to reach the best Hg signal was around 10 % w/v. By the other hand, the concentration of HCl was found to be optimal at levels around 1.0 mol L^{-1}.

3.2.2 Effect of sample loop volume

Additionally, we studied the influence of the sample loop volume upon the Hg signal as peak height. As can be observed in Figure 2, the mercury signal increased proportionally to the sample loop volume up to 100 µL. At higher loop volumes, the signal remained almost constant. For further experiments, a 100 µL-sample loop was employed.

3.3. Calibration curves and analytical performance

Mercury was determined in all samples by the standard addition method. Each standard series consisted in three concentrations and triplicate measurements were made in each case. The calibration plots covered a concentration range from 1.0 µg L^{-1} to 15 µg L^{-1} Hg content in the slurries approximately (base value + added value). (DANTE)

The precision was evaluated as the average relative standard deviation (RSD) at a mercury level of 4.0 µg L^{-1} in the slurry, obtaining a RSD of 3.9 %. The detection limit was determined by making 10 replicate measurements with blood serum slurries. Applying the 3s concept of calculation, detection limit turned to be of 25 ng L^{-1} (ppb).

The accuracy was verified through a recovery study applied on two of the samples analyzed (Table 2). Additionally, the proposed methodology was applied to these samples with a total microwave-assisted digestion. Table 3 shows the results obtained by the two

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samples pretreatment. The results were compared and no significant differences were observed.

Unfortunately, we have no standard reference materials with a certified content of mercury. In this case, a recovery study can be considered as a validation alternative. Although this practice should be avoided, in general, because it disregards the nature of the chemical species in which the element is present, is an alternative to estimate at least the accuracy. [24]

Table 4 compares the analytical performance of this method whit others related works published recently.

4. Conclusions

The developed procedure offers an accurate alternative to those based on a previous complete digestion of samples for the determination of Hg in biological samples. The method, based on the excellent sensitivity attainable by AFS is a safe and comfortable methodology for operators.

Slurry sampling reduces the reagent consumption and time of analysis, allowing improves in detection limits and simplicity. Soft room-temperature treatment of the samples reduced risks of Hg looses and sample contamination.

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Acknowledgments

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Table 1

Furnace temperature program for Hg determination

Optics	
HCL current	20-100 mA
Observation height	8 mm
Wavelength	253.7 nm
Atomizer	
Atomizer temperature	80-200 ºC
Carrier gas flow rate	400-600 mL min^{-1}
Reducing agent	10% (w/v) SnCl$_2$ in 10% (v/v) HCl
Acid concentration in sample	10% (v/v) HCl
Pump speed	Maximum without flooding gas-liquid separator
Performance Data	
Linear working range	From 0.5 μg L^{-1} up to at least 20 μg L^{-1}
Detection limit	0.025 μg L^{-1}

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Table 2

Concentrations of Hg in human serum blood.

Sample	Base ($\mu g\ L^{-1}$)	Hg Added ($\mu g\ L^{-1}$)	Hg Found ($\mu g\ L^{-1}$)	Recovery (%)[a]
Patient 1	1.5	0	1.5±0.1	-
	1.5	4.0	5.5±0.1	100
	1.5	8.0	9.4±0.1	98.7
Patient 2	5.8	0.0	5.8±0.1	-
	5.8	4.0	9.7±0.2	97.5
	5.8	8.0	13.6±0.1	97.5

[a] [(Found - base)/added] x 100

Table 3

Mercury determination in two patients through the proposed salurry sampling-AFS and total microwave digestion-AFS. (n =3, 95% confidence level)

Sample	Microwave digestion	Slurry sampling
Human serum*	8.71 ± 0.15 µg L^{-1}	8.82 ± 0.20 µg L^{-1}

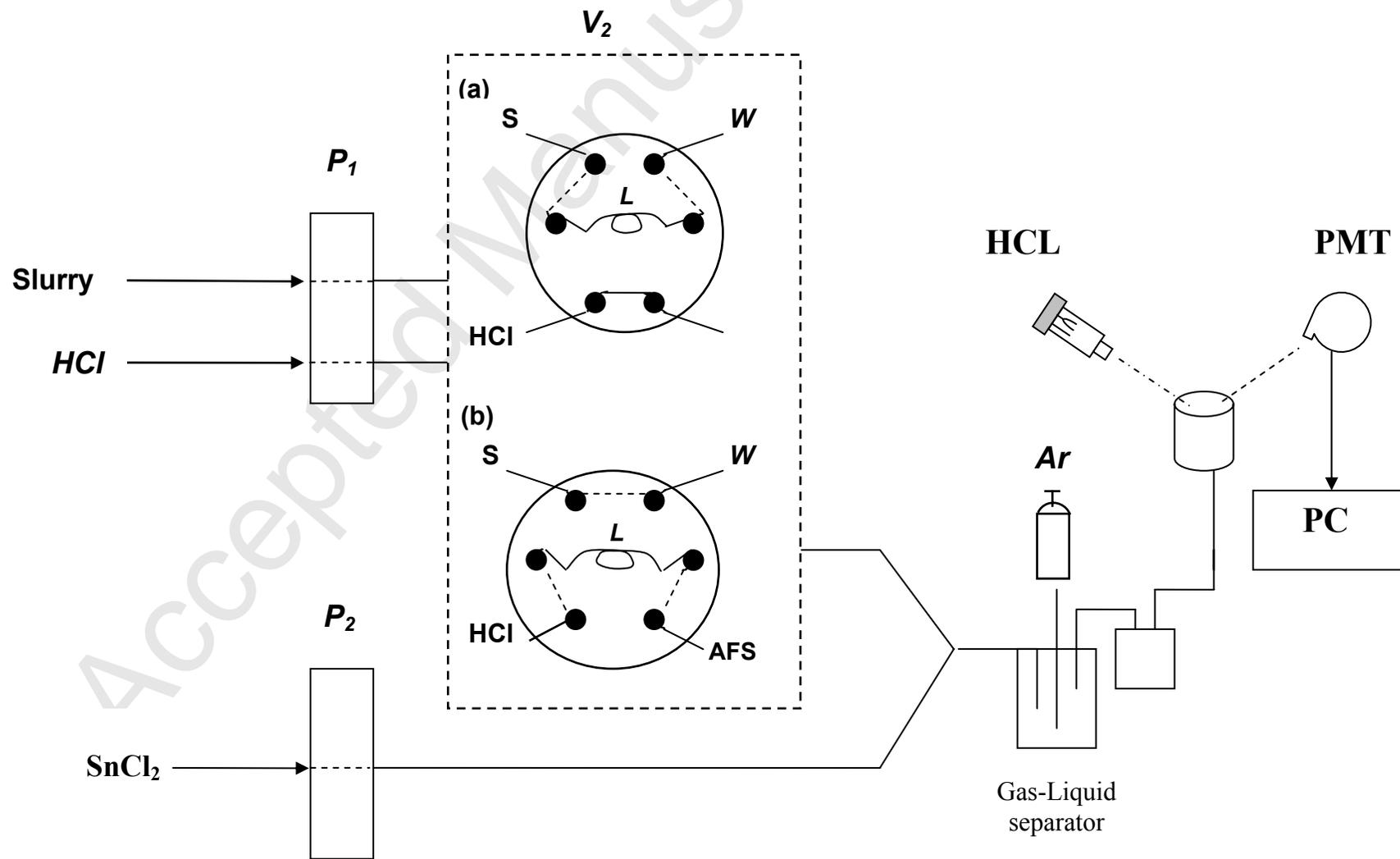*Pool of serum obtained from ten voluntary patients

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Table 4

Comparison among this method and other related methods for mercury determination in
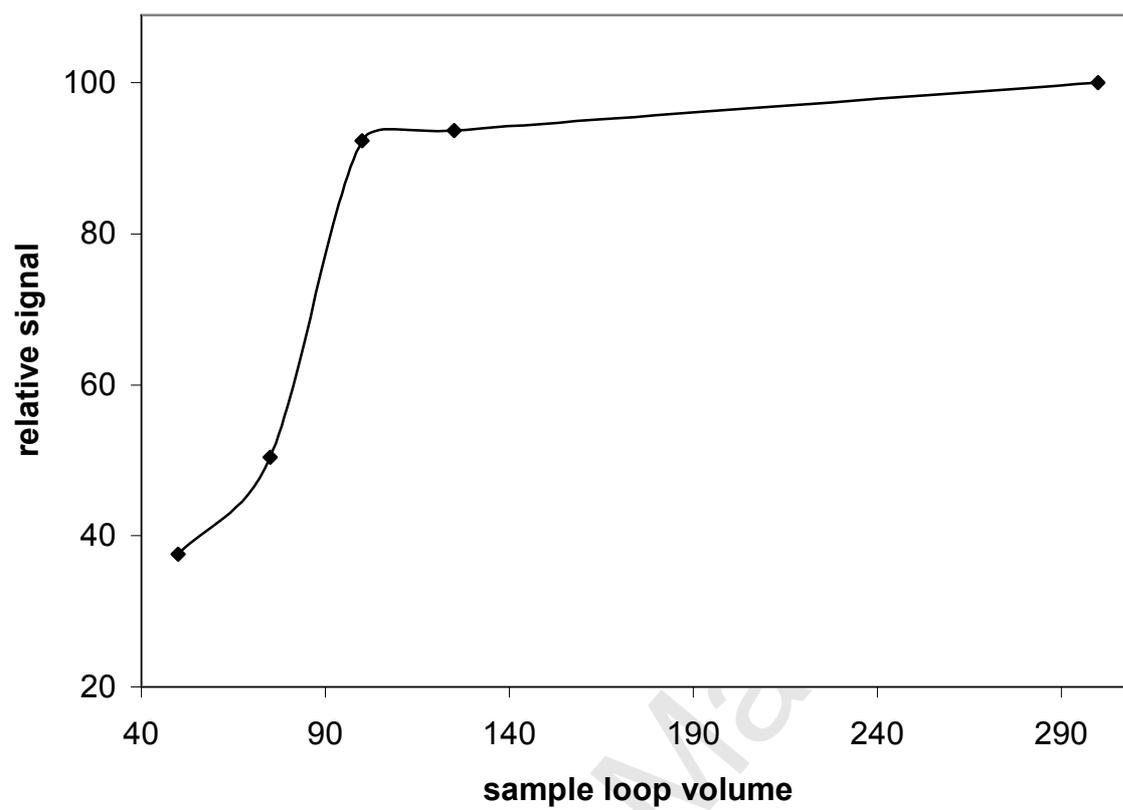
biological samples

Type of samples	Detection Limit	Relative Standard Deviation (%)	Sample Amount	Technique	References
Serum blood	0.025 µg L^{-1}	3.9	1 ml	CV-AFS	This work
whole blood	0.2 µg L^{-1}	<10	0.3 ml	FI-CVAAS	[7]
Biological samples	0.01 µg L^{-1}	4	0.2 ml	GFAAS	[11]
milk	11 µg g^{-1}	3.4	1 g	CV-AFS	[13]
Hair human	0.2 µg g^{-1}	-	0.01 mg	AFS	[25]

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CAPTIONS OF FIGURES

Figure 1. Schematic diagram of the instrumental setup. **P1** and **P2**, persitaltic pumps; **S**, sample path; **W**, waste; **L**, sample loop; **V**, six port rotary valve; **HCL**, hollow cathode lamp (radiation source); **PMT**, photomultiplier (detection system); and **PC**, personal computer (data acquiring, processing and registering). **(a)** load position and **(b)** injection position.

Figure 2. Efect of the sample loop volume upon the mercury signal.

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