Determination of methylmercury and inorganic mercury in whole blood by headspace, cryofocusing gas chromatography and atomic absorption spectrometry

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A method for the determination of different mercury species in whole blood is described. Inorganic mercury (InHg) was determined in 2 ml of standard solutions or blood samples using head space (HS) injection coupled to atomic absorption spectrometry (AAS) after treatment with concentrated sulfuric and tin(II) chloride as a reductant agent in a closed HS vial. After stirring, the InHg was converted to elementary mercury and carried with a nitrogen flow through a quartz cell heated at 200 °C and the absorbance signal was evaluated by AAS. For the determination of methylmercury (MeHg), 2 ml of a standard solution or a blood sample were treated with 10 mg of iodoacetic acid and 0.4 ml of concentrated H_2SO_4. Then, the MeHg species were HS-injected into a gas chromatograph (GC), separated on a semicapillary column (AT-1000) with a flow of helium, then carried to the quartz cell heated at 1000 °C and detected by AAS. The high content of salts in blood samples, where sodium chloride is the major component (0.14 mol l^{-1}), affected the gas–liquid distribution coefficient of both mercury species in the HS vial. A linear calibration graph was obtained in the ranges 1–20 and 1–125 μg Hg l^{-1} added as InHg and MeHg, respectively. The detection limits for InHg and MeHg were 0.6 and 0.2 μg Hg l^{-1}, respectively. The relative standard deviations for eleven independent measurements were 5% for both mercury species. Recovery values ranging from 98 to 106% for InHg and from 95 to 105% for MeHg and from 93 to 95% for ethylmercury (EtHg) were obtained. The accuracy of the proposed method was also established by the analysis of certified whole blood samples for InHg and MeHg. No difference between the sum of these two species determined by our procedure and the recommended total mercury concentrations in the certified samples was observed. Results for the determination of MeHg and InHg in 30 controls and 30 dentists are presented to illustrate the practical utility of the proposed method.

Introduction

Mercury is widely distributed in nature, circulating among several media and occurring in different chemical forms, which show various degrees of toxicity.[1,2] In addition, it has been recognized that InHg compounds are converted into more toxic organomercury (OrHg) compounds by living organisms during natural cycles.[3] Among the OrHg, MeHg is the most predominant and dangerous species, which may be present in biological samples. Particularly, MeHg is the dominant species in human whole blood; *e.g.*, some authors[4] have previously reported 4.64 ± 2.32 and 1.67 ± 1.45 μg Hg l^{-1} as MeHg and InHg, respectively. Therefore, there is a need to distinguish between InHg and OrHg compounds in environmental and biological materials.

Currently, the methods of choice for mercury determinations in human whole blood at trace levels are based on cold vapor atomic absorption spectrometry (CVAAS),[5–8] where Hg(II) is chemically reduced to elemental mercury in the presence of a reducing agent. Following a prior conversion of all OrHg species into InHg, total mercury can be determined along with the original InHg present in the sample, so that OrHg is determined by difference. A bewildering variety of combinations of strong acids, oxidants, UV irradiation, and elevated temperatures have been used and recommended for off-line decomposition of OrHg in different matrices.[9–12] InHg can be determined after reduction with Sn(II) without prior digestion if sufficient levels of Cd^{2+} are added to the reaction medium.[4] While sodium borohydride ($NaBH_4$) is often used for the determination of total mercury,[13] InHg and MeHg,[13–16] it is worthwhile to point out that some workers reported that $NaBH_4$ does not equally reduce all OrHg compounds to elemental mercury.[9] Total mercury in human whole blood samples has been recently determined by inductively coupled plasma mass spectrometry (ICP-MS)[17] and also by electrothermal atomic absorption spectrometry (ETAAS) following extraction in toluene of the complexes of mercury species with diethyldithiocarbamate and a back-extraction into dilute hydrochloric acid in order to decrease the background absorbance.[18] In recent years on-line sample pre-treatment is of particular interest for mercury determination due to the risk of sample contamination, analyte volatilization and adsorption losses during extensive off-line sample digestion procedures. Now, a wide variety of different online procedures have been carried out by flow injection (FI) techniques and treatment with microwave radiation providing a safe, contamination-free closed sample handling system.[9,19–25] On the other hand, hyphenated techniques are nowadays preferred for mercury speciation. Gas chromatography (GC) is the most popular separation technique employed and it has been successfully coupled to different detection techniques such as electron-capture detection (ECD),[26,27] AAS,[28,29] microwave-induced plasma-atomic emission detection (MIP-AES),[4,30–32] glow discharge-AES (GD-AES),[33] ICP-AES,[34,35] ICP-MS[36] or atomic fluorescence spectrometry (AFS).[37] Liquid chromatography (LC) coupled to different types of detectors such as: AAS,[38] MIP-AES,[39] direct current plasma (DCP-AES),[40] and ICP-MS[22] has also proved suitable for the determination of different mercury species. Sometimes,

these methods need a long time sample preparation, or give too high detection limits. On the other hand, frequently, due to the low levels of the mercury compounds in some matrices, a preconcentration step is also required before the GC separation. Normally, this step has been accomplished on a resin containing dithiocarbamate[41,42] or sulfhydryl groups,[43] on a Tenax mini-column[37] or by cryofocusing techniques.[29]

The HS technique uses the equilibrium between the volatile components of a liquid or solid sample and the surrounding gas phase in a sealed vessel. Aliquots of the gas are then removed by an inert gas usually for GC analysis. Thus the HS technique is a convenient way to separate volatile analytes from the bulk of the matrix, providing a proper selection of the equilibrium conditions. The concentration of the volatile compounds can be enhanced in the head space, facilitating in this way the determination of traces of analyte in the sample.[44,45] Some workers have used the HS technique coupled to GC with MIP detection to analyze OrHg compounds in biological samples.[30] The advantages of such a combination are its simplicity, it avoids the "column performance" degradation,[26] all the reaction steps take place in the head space vial,[31] and it effectively separates the analyte from the interfering matrix.

In this work we propose the use of HS directly coupled to AAS for the determination of InHg or coupled to GC and thereafter to the AAS for the determination of OrHg. In this way, the determination of different mercury species in whole blood samples is possible. The sensitivity for the determination of OrHg species was improved by means of online cryofocusing technique.

Experimental

Instrumentation

All the absorbance measurements were carried out with a Perkin-Elmer Model 3100 atomic absorption spectrophotometer (AAS) equipped with a Perkin-Elmer Hg hollow cathode lamp operated at a current of 5 mA. The wavelength was set at 253,7 nm and the slit width was 0.7 nm. A quartz absorption cell, 175 mm long and 15 mm diameter inlet with quartz windows was used.

For the InHg determination, a Perkin-Elmer HS sampler model HS-40 equipped with vials of 22 ml was connected through a capillary fused silica transfer line to a quartz cell heated at 200 °C. A stream of dry and pure nitrogen at a pressure of 32 psi was used to carry the volatile compounds formed in the HS vials to the quartz cell of the AAS.

For the OrHg determination, the HS was mounted on a Perkin-Elmer model AutoSystem gas chromatograph equipped with a 30 m × 0.53 mm id fused-silica semicapillary open tubular (FSOT) column AT-1000 coated with 1.2 μm film thickness of polyethylene glycol ester (Alltech) with He as a carrier gas at 14 ml $min.^{-1}$ Another capillary fused silica transfer line from the GC column outlet was connected to the quartz cell of the AAS heated at 1000 °C. To preconcentrate the analytes, a cryofocusing and a water adsorption trap, commercial accessories of the HS 40 were used.

Reagents and samples

Unless otherwise stated, all chemicals used were of analytical-reagent grade. Deionized doubly distilled water and ACS reagent-grade sulfuric, nitric, iodoacetic and hydrochloric acids (Merck, Elmsford, NY, USA) were used throughout. A stock solution (1000 mg Hg l^{-1}) of InHg was prepared by dissolving 1.3535 g of mercury(II) chloride (Merck, Elmsford, NY, USA) in 1 l of a solution of 5% (v/v) nitric acid (65% w/v) in water. Working standards were freshly prepared by appropriate dilution with the same solution. Tin(II) chloride reducing agent was prepared fresh daily by dissolving 4 g of tin(II) chloride dehydrate (98% w/w from BDH, Poole, Dorset, UK) in 10 ml of concentrated hydrochloric acid (37% w/v) to give a final concentration of 40% w/v. Aqueous standard solutions of MeHg or EtHg chlorides (1000 mg Hg l^{-1}) were prepared by dissolving appropriate amounts of the salts (both 95% w/w from Johnson-Matthey GmbH, Karlsruhe, Germany) in 5 ml methanol (Merck, HPLC grade) and diluted to volume with Milli-Q-water (Millipore, Milford, MA, USA).

Due to the fact that in preliminary studies a strong saline effect was observed, (see below), all standard mercury solutions were also prepared in 0.14 mol l^{-1} sodium chloride (99.9% w/w NaCl from Prolabo, Paris, France). All stock standard solutions were stored in the dark and refrigerated in acid-washed, stoppered glass calibrated flasks and diluted to yield working standards at the concentrations of interest.

The gases used in the HS-GC-AAS system (He, Ar, N_2) were of SR-grade.

Sample collection

In order to use a representative blood sample with the same composition in all the experiments described in this study, a pool of whole blood (*ca.* 0.5 l) was obtained by mixing portions of *ca.* 10 ml of samples from 50 adults with no known record of occupational exposure to mercury compounds. Whole blood samples were also obtained from two study groups, the control and dentists groups both included 30 subjects. The blood was drawn from the vein of the forearm of each subject using 10 ml disposable syringes with needles and inmediately transferred to the respective vessels containing heparine as anticoagulant and kept under refrigeration (4 °C).

Two lyophilized human whole blood samples (Seronorm Batches 905 and 906, Nycomed AS, Oslo, Norway) having recommended total mercury concentrations, were used for assessing the accuracy of the analytical procedure.

Procedure

The proposed method comprises two steps, one for the analysis of InHg and the other for OrHg species as detailed below.

Determination of InHg. Initially, 0.25 ml of concentrated sulfuric acid and 0.4 ml of a tin(II) chloride reducing agent solution were added to each HS vial containing 2 ml of standard aqueous mercury solution (for calibration purposes) or 2 ml of whole blood samples. The mixture was mechanically stirred for 2 min with a Vortex (HEIDOLPH, Germany) and then thermostated at 65 °C for 3 min. During the termostatization step, the HS system was in 'standby' position (Fig. 1) and a carrier stream of nitrogen at a pressure of 32 psi flowed through the transfer line maintained at 150 °C, to the quartz cell. Afterwords, the HS was programmed in the "pressurisation" position and the HS sampling needle preheated at 150 °C descended, pierced the septum cap of the sample vial and the vial was pressurized with nitrogen at 32 psi for 3 min. At the end of this step, the flow of nitrogen was stopped, the vapor from the HS vial flowed for 24 s through the transfer line to the atomizer, and the absorbance signal evaluated by AAS.

Determination of MeHg. In this case, 2 ml of a standard solution of MeHg or of a whole blood sample were placed in the HS vial. Then, 10 mg of iodoacetic acid and 0.3 ml of concentrated sulfuric acid were added. The vial was vigorously shaken for 1 min and thermostated for 6 min at 110 °C. After a pressurization time of 2 min, the overpressured gas sample in the HS vial was applied for 24 s (sampling time) onto the GC

column through the transfer line maintained at 150 °C. During the sampling time, the cryofocusing on the column by liquid nitrogen permitted the head column to reach a temperature −40 °C. Then, the GC oven was programmed in order to provide a good separation of the MeHg species that emerged from the column to the quartz cell set a 1000 °C and the absorbance signal was evaluated. The capillary fused silica transfer line from the column to the detector was termostated at 170 °C. At the end of the GC column a makeup of Ar at 60 ml min^{-1} before continuing to the detector was necessary to avoid broadening of the signals.

Results and discussion

Optimization of experimental conditions

The operating conditions for the determination of InHg and MeHg species were optimized and are given in Tables 1 and 2, respectively. The one-factor-at-a-time method was used to optimize the experimental parameters, while the rest were kept constant.

It is known that matrix components in the liquid phase, in particular the salt concentration, affect the HS behavior of volatile substances.[46] The magnitude of this effect depends also on the distribution coefficients ($K_D = C_g / C_l$, where C_g and C_l are the concentrations of the analyte in the gas and liquid phase, respectively). Blood samples have a high content of salts where NaCl is the major component at a concentration of 0.14 mol l^{-1}.[47] To evaluate this effect, a study was undertaken to assess the analytical characteristics of the system by preparing solutions of the analytes (InHg and MeHg) in water and in NaCl 0.14 mol l^{-1}; also blood samples were spiked with InHg and MeHg. The salting-out of standard solutions affected the gas–liquid distribution of mercury species in the HS vials (see below). On this basis calibration graphs for the determination of InHg and MeHg were prepared in solutions of 0.14 mol l^{-1} of NaCl.

Optimization of conditions for InHg determination

The effect of different sample volumes in the HS vial was examined. The sensitivity of the analysis improved as the volume of the sample increased for InHg in an aqueous solution, due to a concomitant increase of the amount of the analyte and to a decrease in the HS volume.[44] For whole blood samples the absorbance reached a maximum at 2 ml decreasing at higher sample volumes. This last effect is probably due to the fact that at the optimal thermostating temperature (see below) blood samples clot at volumes above 3 ml. A sample volume of 2 ml was fixed for all further analysis.

Tin(II) chloride in concentrated hydrochloric acid as reducing agent was used to reduce Hg(II) to Hg°. This reagent has the advantage that in untreated samples only InHg is reduced under acidic conditions.[8,11] The effect of the reductant volume on the absorbance was examined in the range from 0.1 to 0.5 ml. The results in Fig. 2 show that in either case, better sensitivities were observed for 0.4 ml of 40% w/v tin(II) chloride solution for the determination of InHg in aqueous standard solutions (with and without the addition of sodium chloride at a concentration of 0.14 mol l^{-1}) and in blood samples.

The stirring time of the sample with the reducing agent is necessary to allow a complete reaction and to prevent the entrapment of the volatile analyte within the matrix through homogenization during the extraction procedure. This parameter was studied in the range from 0 to 3 min. The absorbance signal reached a plateau after 2 min which was considered to be optimum time needed to attain an equilibrium.

The addition of concentrated sulfuric acid was necessary in order to avoid the clotting of blood samples during the termostating step.[8] On the other hand, the appreciable heat generated during the dilution of concentrated sulfuric acid in water helps to reduce the solubility of the analyte and to increase its vapor pressure in the HS vial.[48] The volume of concentrated sulfuric acid was varied in the range from 0 to 0.4 ml (Fig. 3). A volume of 0.25 ml permitted to obtain a good signal with high recovery values (Table 4).

Higher thermostating temperatures increased the sensitivity of the analysis. This effect could likely be due to an enhanced concentration of Hg° in the gas phase owing to higher K_D of this volatile species.[45] The absorbance signal increased up to 65 °C and reached a plateau at higher temperature due to an improvement on the kinetics of the generation and stripping of mercury vapor from the solution. However, temperatures above 80 °C led to the condensation of water vapor at the end windows of the quartz cell giving a low reproducibility.[9,11] No significant variation on the signal was observed for thermostating times between 3 and 10 min because the gas–liquid equilibrium is reached in this temperature range. Therefore, for futher studies, the vials were thermostated at 65 °C for 3 min.

In order to transfer the Hg° present in the HS, the vapor pressure into the vial was not sufficient to permit the analyte to reach the cell and to produce a measurable signal. The best signal was obtained when the vial was pressurized with nitrogen at 32 psi for 3 min.

The effect of the quartz cell temperature on the absorbance signal has been studied. The results showed that with a temperature of 200 °C the reproducibility and the sensitivity

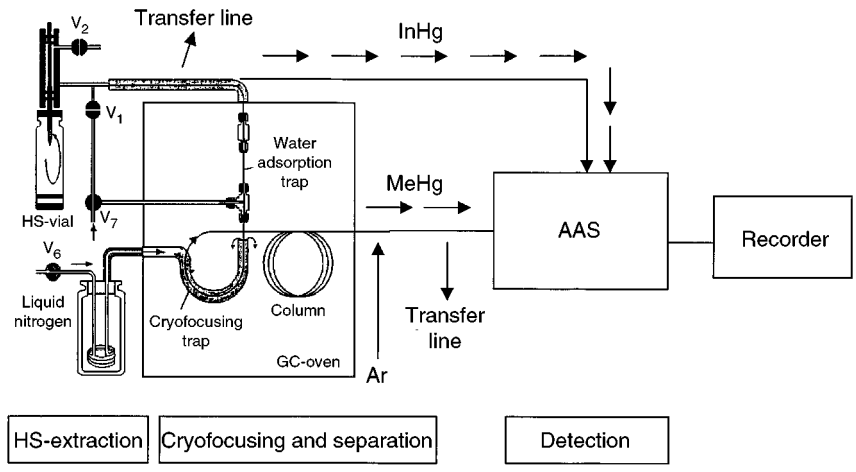

Fig. 1 Schematic diagram of the system used for the determination of mercury species in whole blood samples.

were improved. Temperatures higher than 200 °C decreased the signal, probably because the residence time of the mercury vapor in the cell decreased and a low precision was obtained.

The sampling time of the analyte from the HS to the detector was varied from 0.1 to 0.6 min. The signal increased as the sampling time increased; however, non-reproducible and irregular signals with tailing were obtained for sampling time values higher than 0.4 min.

The optimum experimental parameters for the determination of InHg are summarized in Table 1.

Table 1 Optimized operating conditions for the determination of InHg in blood by HS-AAS

Component	Parameter	Value
Chemical	$SnCl_2$ in concentrated HCl	40% w/v
HS	Sample volume	2 ml
	Sulfuric acid volume	0.25 ml
	$SnCl_2$ volume	0.4 ml
	Termostatization time	3 min
	Termostatization temperature	65 °C
	Pressurization time	3 min
	Agitation time	2 min
	Carrier gas (N_2) pressure	32 psi
	Transfer line temperature	150 °C
	Needle temperature	150 °C
	Injection time	0.4 min
Detector	Slit width	0.7 nm
	Wavelength	253.7 nm
	Lamp current intensity	5 mA
	Quartz cell temperature	200 °C

Table 2 Optimized experimental conditions for the determination of MeHg in blood by HS-GC-AAS

Component	Parameter	Value
HS	Sample volume	2 ml
	Termostatization time	6 min
	Termostatization temperature	110 °C
	Pressurization time	2 min
	Agitation time	1 min
	Sulfuric acid volume	0.3 ml
	Amount of iodoacetic acid	10 mg
	Needle temperature	150 °C
	Injector transfer line temperature	150 °C
	Pre-cryogenic time	2 min
	Post-cryogenic time	0.6 min
	Pressure of coolant gas (N_2)	580 psi
	Injection time	0.4 min
GC	GC oven temperature programme	100 °C for 1.5 min, to 170 °C for 10 min)
	Heating speed	15 °C min^{-1}
	Injector temperature	150 °C
	He flow rate	14 ml min^{-1}
	Transfer line temperature	170 °C
Detector	Slit width	0.7 nm
	Wavelength	253.7 nm
	Lamp current intensity	5 mA
	Makeup Ar flow rate	60 ml min^{-1}
	Quartz cell temperature	1000 °C

Table 3 Regression equations of absorbance signal obtained in different matrices

Analyte	Matrix	Regression line[a]	*r*
InHg	Water	A = 0.0007 + 0.0122 C	0.9998
	NaCl 0.14 M	A = 0.0005 + 0.0056 C	0.9990
	Blood	A = 0.0008 + 0.0057 C	0.9987
MeHg	Water	A = 0.0003 + 0.0155 C	0.9993
	NaCl 0.14 M	A = 0.0006 + 0.0122 C	0.9988
	Blood	A = 0.0007 + 0.0123 C	0.9989

[a] A and C indicate absorbance and mercury concentration in μg l^{-1}, respectively. The concentration ranges were from 1 to 20 and from 1 to 125 μg Hg l^{-1} for InHg and MeHg, respectively.

Optimization of conditions for OrgHg determination

The variables of the system used for the determination of MeHg species that had to be optimized were split in three areas: (i) headspace extraction; (ii) chromatographic separation and (iii) detection.

An attempt to optimize the thermostating temperature of the vials containing MeHg, showed that the absorbance signal increases with an increase in temperature. A thermostating temperature of 110 °C was chosen as a suitable compromise between a good sensitivity and excessive formation of water vapour which are known to affect the stationary phase of the column [31] and the precision of the results. On the other hand, the

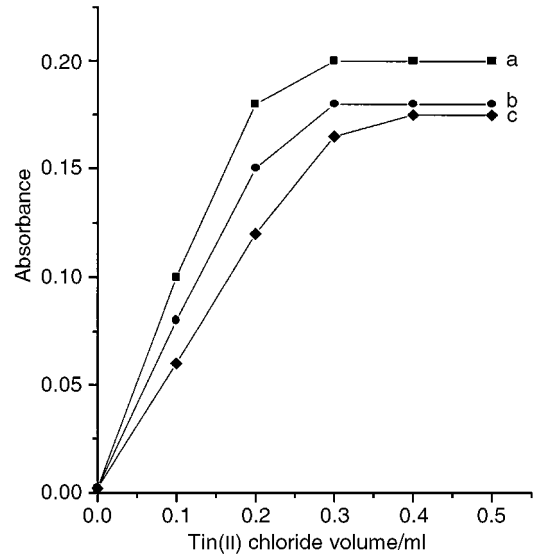

Fig. 2 Effect of the volume of tin(II) chloride solution in the HS vial on the absorbance signal. 5 μg of Hg l^{-1} as InHg in: (a) water, (b) sodium chloride 0.14 mol l^{-1} and (c) blood sample. Conditions as in Table 1.

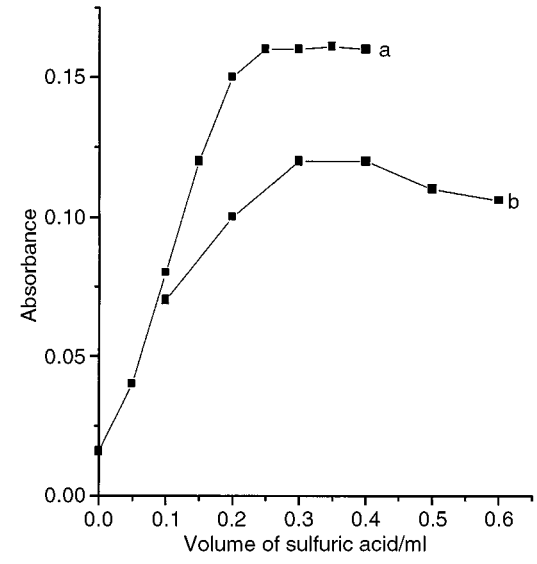

Fig. 3 Effect of the volume of concentrated sulfuric acid in the HS vial on the absorbance signal, from: (a) 5 μg Hg l^{-1} as InHg and (b) 20 μg Hg l^{-1} as MeHg. Other conditions as specified in Tables 1 and 2.

Table 4 Recovery of mercury added to pooled blood sample

	Added/μg Hg l^{-1}			Found/μg Hg l^{-1}			Recovery (%) ± RSD (n = 3)		
Sample	InHg	MeHg	EtHg	InHg	MeHg	EtHg	InHg	MeHg	EtHg
1	2.5	0	0	2.65	—	—	106 ± 4	—	—
2	5	0	0	4.95	—	—	99 ± 2	—	—
3	10	0	0	9.80	—	—	98 ± 2	—	—
4	—	10	20	—	10.5	19	—	105 ± 2	95 ± 2
5	—	20	30	—	19	28	—	95 ± 1	93 ± 3
6	5.0	20	30	5.1	21	29	102 ± 2	105 ± 2	97 ± 3
No spiked blood				1.6	2.8	LOD			

equilibrium between the gas and liquid phases for all species was reached in 6 min, therefore, this value of thermostating time was fixed for subsequent analysis.

The MeHg present in biological samples is essentially bound to protein sulphydryl groups, therefore a cleaving agent is necessary in order to liberate the MeHg species.[30] It was found that concentrated sulfuric acid in combination with iodoacetic acid, completely released the MeHg from tuna fish tissue samples through the formation of the corresponding, more volatile, iodide form.[30] Based on these considerations, in this work, the same cleaving agents were used in order to achieve the whole extraction of the MeHg species from whole blood samples.

The effect of the amount of each reagent added to 2 ml of whole blood pipetted in the HS vial containing 20 μg of Hg as MeHg l^{-1} and 10 mg of iodoacetic acid was examined. The volume of concentrated sulfuric acid was varied in the range from 0.1 to 0.6 ml. The vials were vigorously shaken for 1 min and treated as described in the procedure section. From the results shown in Fig. 3 it can be concluded that a maximum signal is observed for 0.3–0.4 ml of sulfuric acid. For volumes higher than 0.4 ml, the signal decreases probably due to the formation of the less volatile MeHg sulfate salts.[30] The influence of the amount of iodoacetic acid is shown in Fig. 4; the best signal was obtained for an amount of 10 mg of iodoacetic acid.

It is known that in the case of samples with large K_D the influence of the sample volume on the concentration of analytes in the HS is significant.[44] MeHg has a relatively large K_D measured in water.[49] The effect of the sample volume on the absorbance was studied for aqueous standards and for blood samples spiked with MeHg. The sample volume was varied between 1 to 5 ml. For 5 ml of aqueous standard solution, the concentration of MeHg in the headspace was 4 times higher than that found for 1 ml. A different behavior was found for blood samples. The signal increased with increasing the sample volume up to 2 ml, and thereafter decreased for volumes higher than 4 ml. This effect is due to the fact that at the thermostating temperature the blood samples clot. On the other hand, if the sample volume is increased, then the time of thermostatization must also increase due to the increased diffusion path length in the liquid phase.[44] A sample volume of 2 ml was found to be a good compromise between sensitivity, precision and time of analysis.

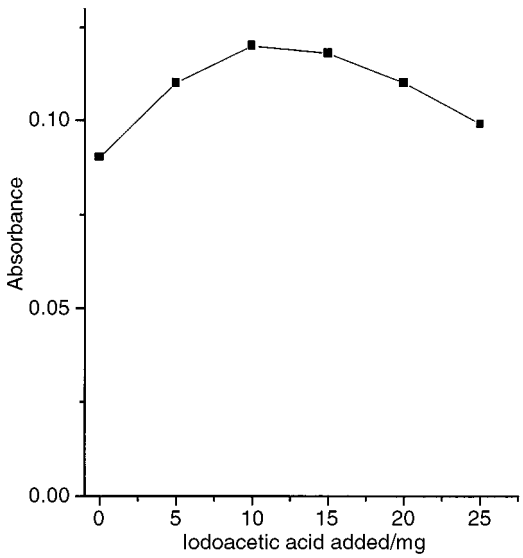

Fig. 4 Effect of the amount of iodoacetic acid for the determination of Hg added as MeHg. Conditions as in Table 2.

When HS-GC utilizing capillary or semicapillary columns are used for trace analysis, sample enrichment is often needed.[50] This involves a relatively long sampling time. As a result, the signals from the analytes may be too broad causing peak distortion and poor resolution. In order to avoid this inconvenience, the capillary head trapping technique was employed.[50] This was accomplished by jacketing about 30 cm of the first portion of the column with a PTFE tubing through which a pre-cooled nitrogen gas in a liquid nitrogen bath was conducted at a flow rate of 20 l min^{-1} which flowed against the direction of the carrier gas in the column providing a temperature gradient inside the capillary column. The nitrogen flow is switched on and off by means of a solenoid valve fitted to the HS 40 which is started prior to sample injection. The cooling effect at the column inlet is dependent on the pressure of the coolant gas (N_2). This parameter was optimized and with a presure of N_2 of 580 psi the head column cooled down to −40 °C. On the other hand, the parameters involved in the optimization of the cryofocusing time are the pre- (before and during sample injection) and post- (after sample injection) cryofocusing times. The cryofocusing time was also optimized and the best values for the pre- and post- cryogenic times in order to permit a sampling time of 24 s without broadening signal of analytes were 0.6 and 2 min respectively. On the other hand, blood samples contain water which at the low temperature of the trap freezes and may break the column head. In the experimental arrangement described in this work (Fig. 1), the water vapour generated at the thermostating step does not produce any problems because an online water adsorption trap was installed prior to the cryogenic headspace trapping. This trap is an accessory of the HS autosampler of unknown composition. The recovery tests carried out in this work (see below) evidenced no signs of adsorption of the volatile mercury species, indicating the inertness of the adsorbing material. Using this cryofocusing technique an improvement in the sensitivity by a factor of 20 was obtained.

A good separation of MeHg and EtHg was obtained using an injection temperature of 150 °C, a helium flow rate of 14 ml min^{-1} and a chromatograph oven temperature programmed to start at 100 °C for 1.5 min and then increased up to 170 °C at a rate of 15 °C min^{-1} and held for 10 min at this temperature. The individual species which emerged from the column were mixed with a makeup gas (Ar) before continuing to the quartz cell. The flow rate of the makeup was optimized in order to obtain narrow

and reproducible signals. The best results were obtained for an Ar flow rate of 60 ml min^{-1} . On the other hand, the cell temperature was fixed at 1000 °C, which is suitable for breaking the organomercury compounds down to elemental mercury.[37] Under these conditions we obtained typical chromatograms (Fig. 5) for water standard solutions of MeHg and EtHg and for blood samples spiked with both analytes, respectively. EtHg was also spiked to blood samples because it may be present after direct exposure to the compound.

Analytical figures of merit

Table 3 shows the regression equations of the absorbance as a function of mercury species concentration obtained for standard mercury species solutions prepared in water, in sodium chloride 0.14 mol l^{-1} and from spiked blood samples. These data were obtained by running calibration curves using the method of standard additions. Each calibration set included seven data points and each point was run at least three times. For all mercury species (InHg and MeHg) the slopes of the calibration graphs for blood and salted solutions were essentially the same. However, the slope of the graph for aqueous solutions was higher than the others. These results are in agreement with those previously found by Burguera *et al.*.[51] for the determination of iodide in urine samples. The magnitude of the salting-out effect depends on the amount of salt added and the partition coefficient of the system.[46,52,53] On the other hand, in the case of MeHg species, the presence of salts in the sample reduce the K_D due to its polarity, making it harder to separate from the sample matrix.[46] The fact that the slopes of the calibration curves prepared with blood samples and in 0.14 mol l^{-1} sodium chloride solutions were the same, may suggest that the observed matrix effect could be mainly due to salt concentration. This means that sodium chloride 0.14 mol l^{-1} calibration is a real possibility and this type of calibration was therefore used in subsequent experiments.

The reproducibility of this method measured at 5 μg Hg l^{-1} added as InHg and at 10 μg Hg l^{-1} added as MeHg in blood samples were in either case of 5% (n = 11). The detection limits of this method (defined as the concentration yielding a peak absorbance three times the standard deviation of the blank (n = 5)) were 0.6 μg InHg l^{-1} and 0.2 μg MeHg l^{-1}.

The recovery values of InHg and OrHg (added as MeHg and EtHg) from spiked blood samples are shown in Table 4. These ranged from 98 to 106 for InHg, from 95 to 105 for MeHg and from 93 to 95 for EtHg. In order to verify that the proposed procedure permits the speciation of all mercury compounds, the blood samples were also spiked with mixtures of the standards. The recovery values obtained for InHg, MeHg and EtHg were 102, 105 and 97%, respectively (Table 4). These results proved that by using this method, the determination of mercury (added as Ing and MeHg) in blood samples is possible.

The accuracy of the proposed method was further tested by analyzing lyophilized human whole blood (Seronorm batch 905 and 906) samples, having known total mercury concentrations. It should be pointed out that in the production of Seronorm 905 and 906, InHg was added as Hg^{2+} to give supplementary concentrations of 5 and 10 μg l^{-1} Hg. These samples were reconstituted as recommended by the supplier and were analyzed by our procedure the same day. The results listed in Table 5 indicate that in these materials the mercury is present in inorganic and also in organic form. Similar results were previously obtained by Bulska *et al.*[4] in the speciation of mercury in human whole blood by capillary GC with MIP detection following complexometric extraction and butylation.

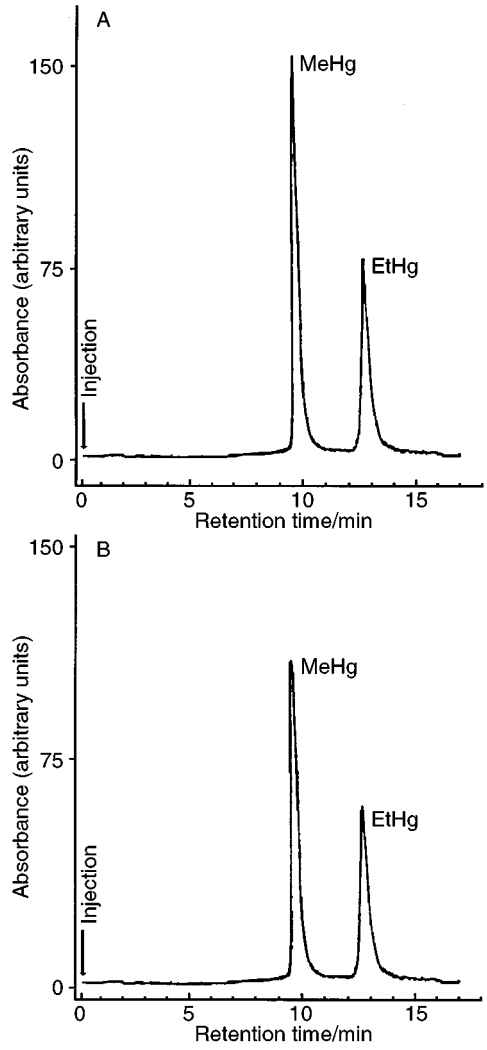

Fig. 5 Two typical chromatograms corresponding to the determination of MeHg and EtHg by HS-GC-AAS. (A) Water standard solutions of 10 μg of Hg l^{-1} added as MeHg and EtHg, respectively; (B) blood samples spiked with 10 μg of Hg l^{-1} added as MeHg and EtHg, respectively. The experimental conditions were as specified in Table 2. The retention times for MeHg and EtHg were 10.0 and 13.0 min, respectively.

Analysis of human whole blood samples

Table 6 shows the distribution of MeHg and InHg in the control and dentists subjects. The Hg content of the dentist group was higher than that obtained from the control group; such differences were statistically significant (P < 0.05). It was also evident that in both groups higher MeHg contents are found, which may be due to others factors such as fish consumption. Our results are in good agreement with previously obtained by others authors.[4]

Table 5 Analysis of reconstituted Seronorm lyophilized human whole blood samples

Seronorm Batch No	Measured value/μg Hg l^{-1} [a] InHg	MeHg	Total mercury/μg l^{-1} [b]	Recommended value/μg Hg l^{-1}
905[c]	5.9 ± 0.8	4.4 ± 0.5	10.3 ± 0.8	10 ± 0.49
906[d]	12.0 ± 0.8	3.5 ± 0.8	15.5 ± 0.8	15 ± 1.1

[a] Each value is the mean ± SD of five measurements. [b] Sum of InHg and MeHg. [c] Spiked with 5 μg Hg l^{-1} as InHg. [d] Spiked with 10 μg Hg l^{-1} as InHg.

Table 6 Results for the speciation of mercury in human whole blood samples from controls and dentists

	Concentration of Hg/μg Hg l^{-1}[a]		
Subjects	Me	InHg	Total mercury
Controls (n = 30)	3.06 ± 0.14	1.52 ± 0.12	3.58 ± 0.13
Dentists (n = 30)	4.00 ± 0.35	2.62 ± 0.30	6.62 ± 0.32

[a] Mean values ± standard deviation.

Conclusions

In conclusion, the developed method is suitable for the determination of InHg and MeHg in whole blood samples. The samples don't need any manipulation prior to automatic treatment and measurement. The reactions take place within the HS vial, which reduces the risk of analytes losses or contamination. The only matrix effect is due to salt concentration and for this reason, the solutions of both mercury species were matrix matched with regard to the sodium chloride content in blood samples. On the other hand, the sensitivity for MeHg species was improved by a factor 20 by using the online cryofocusing technique.

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