Evaluation of Direct Determination of Mercury in Whole Human Blood Using a Mercury Analyzer

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INTRODUCTION

Mercury is a heavy metal which is considered a persistent, mobile, and highly toxic pollutant for human health. It is present in different chemical forms, the more important forms being elemental mercury (Hg), inorganic mercury (Hg²⁺), and organometallic monomethylmercury (CH₃Hg⁺) (1). All of these forms are toxic to humans and animals and most significantly affect the central nervous system. Although there are natural sources of mercury pollution due to volcanoes, forest fires, and weathering of rocks, their contribution is small in comparison to the contribution from anthropogenic sources.

The most important activities related to mercury pollution are fossil fuel combustion processes, solid waste incineration, mining and smelting, and the manufacture of cements and chlorides (2). Atmospheric mercury enters into the hydrosphere and lithosphere through wet and dry deposition. During these processes it is possible to transform inorganic mercury into the most toxic form, CH₃Hg⁺, through biotic or abiotic routes (3). This compound biomagnifies in the aquatic food chain (4,5) and can be completely absorbed in the gastrointestinal tract and thus be transported throughout the body. The consumption of fish, for instance, with high levels of mercury can initiate serious health problems for humans and for wildlife (6).

ABSTRACT

A mercury analyzer (Model SMS 100) was evaluated for the direct determination of mercury in whole human blood samples. The instrument is based on sample combustion in an oxygen-rich furnace, elimination of interferences in a catalytic furnace, mercury concentration by amalgamation with gold, and the release of mercury vapor and its transport in a stream of oxygen to the measurement cell. Mercury determination is performed by atomic absorption spectrometry at 253.7 nm.

In order to obtain complete combustion of the blood sample without loss of mercury, all operating conditions were optimized. The temperatures and times of the combustion step were critical to avoid spluttering of the sample. It was found that the samples of whole human blood, the certified reference material of human blood, and the aqueous standards showed different behavior in this step. The optimized condition for the combustion step was 650 °C for 60 seconds. The temperatures and times for the other steps were also optimized.

The calibration and the analytical performance of the method were also studied. The detection limit obtained for mercury was 0.02 ng, and the relative standard deviations were between 1.1 and 3.2%. The proposed method is an interesting approach to the determination of mercury in whole human blood samples, because sample dissolution with wet digestion methods is not necessary, resulting in the ability to analyze an important number (approximately 7) of samples per hour.

In 2009, the US EPA (United States Environmental Protection Agency) established that mercury is a high priority pollutant (7). In 2008, the EC (European Commission) published a report about the precautions with regard to fish consumption, in particular by children and pregnant women (8). In addition, some organizations are demanding that the FDA (United States Federal Drug Administration) revise its regulation regarding the use of dental amalgams that contain mercury (9).

Although mercury determination can be performed using cold vapor atomic absorption spectrometry, combustion-atomic absorption spectrometry is today routinely used for environmental and biological sample analysis. The advantages of this method are that no sample pretreatment is necessary and the analytical instrument required is inexpensive. The method routinely used is US EPA Method 7473 Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry (10) for samples such as fish tissues (11), environmental and biological samples (12,13), human hair and animal fur (13), crude oil (14), and whole blood (15).

The aim of this work was to evaluate the use of a commercial mercury analyzer for the direct determination of mercury in human whole blood samples using the above-mentioned method.

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EXPERIMENTAL

Instrumentation

The Model SMS 100 mercury analyzer (PerkinElmer, Inc., Shelton, CT, USA) was used for this study. Solid and liquid samples can be introduced with the instrument's autosampler without prior sample preparation. The system uses the principle of thermal decomposition, amalgamation, and atomic absorption and is described in EPA Method 7473 (14) and ASTM Method 6722-01 (15). The schematic of the SMS 100 mercury analyzer is shown in Figure 1.

Reagents and Standard Solutions

All chemicals used were of ultrapure grade. Ultrapure water with a resistivity of $18 \text{ M}\Omega \text{ cm}^{-1}$ (Millipore Corporation, Bedford, MA, USA) was used. The mercury stock solution of 1 mg L^{-1} was obtained from E. Merck, Darmstadt, Germany.

Certified reference materials (CRM) of whole blood, CRM Seronorm™ Trace Elements Whole Blood Level I, Level II, and Level III with a mercury certified content were used and obtained from Sero AS, Billingstad, Norway.

All glassware was soaked for at least 48 hours in 10% nitric acid and rinsed in ultrapure water before use.

Procedure

A 300- μL amount of blood sample was weighed into a nickel sample boat. The boat is introduced by the autosampler into the instrument's combustion tube. This tube is heated in an oxygen-rich atmosphere to release all of the decomposition products. The combustion products are carried in a stream of oxygen to the catalytic section, where components such as halogens, nitrogen oxides, and sulphur are trapped in the catalyzer. This is necessary to avoid interference of these compounds in the determina-

Fig. 1. Schematic of SMS 100 mercury analyzer.

tion of mercury. The remaining vapor, containing the mercury, is transferred to an amalgamation cell that selectively traps the mercury. After elimination of possible residues from the decomposition products using the same stream of oxygen, the amalgamation cell is rapidly heated to release the mercury vapor. This vapor is transferred to an absorbance cell which is situated in the light path of a single atomic absorption spectrophotometer. The absorbance is measured at 253.7 nm with a 25-cm path length cell and for an integration time of 60 seconds.

RESULTS AND DISCUSSION

Optimization of Operating Conditions

In order to optimize the temperature and time of the different steps, a pool sample of whole human blood (300 μL) was used. The initial conditions (as recommended by the manufacturer) were used, but these resulted in spluttering of the sample at the beginning of the decomposition step. This problem can in some cases produce movement of the sample boat outside the autosampler. However, this problem was only observed with whole human blood samples and not with aqueous standards or the whole blood certified reference materials.

Effect of Decomposition Temperature

The effect of decomposition temperature and time was studied. In a first approach using the recommended drying conditions of 300 °C and 45 seconds, decomposition temperatures ranging from 600–800 °C with a decomposition time of 150 seconds were tested. Under these conditions, the spluttering problem remained and, moreover, an important amount of sample residue was observed in the sample boat. Even when the decomposition time was increased to 200 seconds, there was no positive change.

Optimization of Drying Step

The drying step was studied using temperatures ranging from 300 to 400 °C, different times from 240 to 330 seconds, and a decomposition temperature of 650 °C for 200 seconds. Although there was no significant influence of the drying conditions on the absorbance signal, a drying temperature of 400 °C and 300 seconds reduced the spluttering problem considerably.

Effect of Decomposition Time

The effect of decomposition time was studied ranging from 60 to 210 seconds and using a decomposition temperature of 650 °C. Although there were no significant

Fig. 2. Effect of oxygen flow on absorbance profile.

differences in the absorbance signal, we selected 100 seconds as the optimum time. With lower times, incomplete sample combustion was observed, thus, also leaving a sample residue.

Effect of Oxygen Flow

Oxygen flow is a very important parameter for every step of the procedure. The effect of oxygen flow on the absorbance signal was observed at flows between 150 to 450 mL/min. The results obtained are shown in Table I and Figure 2. As can be seen, an increase in oxygen flow produces a decrease in the absorbance signal and shows important variability in the profile peak. The best absorbance peak for best reproducibility was obtained with an oxygen flow of 300 mL/min.

Effect of Catalysis Temperature

The effect of different catalysis temperatures was studied ranging from 500 to 650 °C and using a catalysis time of 60 seconds. To ensure that the catalyzer is at the selected temperature for each blood sample, a blank was also performed. Since no significant differences were observed with different catalysis temperatures, we selected the optimum temperatures as listed Table II.

Fig. 3. Calibration curve using three CRMs. Seronorm™ Trace Elements Whole Blood Level I, Level II, and Level III.

TABLE I
Effect of Oxygen Flow

Oxygen Flow (mL/min)	Absorbance (μ)
150	97,382
250	66,152
300	57,309
350	48,452
400	39,485
450	42,733

The integration time proposed was 60 seconds because it is adequate for the 25 cm long cell path

TABLE II
Optimized Operating Conditions for 300 μL of Whole Blood Sample

Step	Temp. (°C)	Time (s)
Drying	400	300
Decomposition	650	60
Catalysis	600	30
Amalgamation	700	60
Measurement	—	60

which is necessary due to the low levels of mercury usually present in whole human blood samples.

Fig. 4. Comparison of calibration curves.
(●) CRM Seronorm™ Trace Elements Whole Blood Level II only (dotted line).
(×) CRM Level I and Level II (solid line).

Fig. 5. Comparison of calibration curves.
(▲) Aqueous calibration with Hg concentrations of 0, 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 ng (solid line).
(●) CRM Seronorm™ Trace Elements Whole Blood Level II with Hg concentrations of 0, 0.7, 1.8, 2.7, 3.6, and 9.0 ng (dotted line).

ANALYTICAL PERFORMANCE

Calibration

In order to check the linearity of the method, calibration was performed using the Certified Reference Material (CRM) Seronorm™ Trace Elements Whole Blood having three different mercury levels: Level I (2.2 ± 0.2 µg/L), Level II (8.3 ± 1.7 µg/L), and Level III (15.0 ± 1.1 µg/L). To express the

mercury concentrations in micrograms, it is necessary to use the density of the CRM (1060 g/L). Figure 3 shows this calibration, and although there are small deviations for the concentration levels obtained with the CRM Level I and Level III, the correlation coefficient (0.9986) was adequate. It should be noted that when the CRM was used for calibration, a larger amount of sample residue was observed in the

sample boat at the end of the procedure.

We performed calibrations using different amounts of only one certified reference material. For this study, we used different amounts of CRM Level II. Figure 4 shows the calibrations performed with the three CRMs and the curve obtained using only CRM Level II. Since there was no significant difference between the two calibration curves, it is sufficient to use only one CRM.

In order to assess whether it is possible to use aqueous calibrations, we performed a calibration with aqueous standards having mercury amounts ranging from 0 to 20 ng. Different volumes of two mercury standards (20 and 50 µg/L) were used (Table III). Figure 5 is a comparison of the results with the aqueous calibration and the CRM Level II sample. After statistical comparison (95% confidence level, student's *t*-test), it was determined that there is no statistical difference between the slopes of both calibration types. Thus, it is possible to use aqueous calibration because no apparent matrix effects can be observed.

Sensitivity of the Method

The limit of detection (3 SD/m) and the limit of quantification (10 SD/m), where SD is the standard deviation of 11 measurements of a blank and m is the mean slope of the standard addition curve, were 0.02 and 0.05 ng, respectively.

Repeatability of the Method

The repeatability of the overall procedure was assessed by analyzing a real blood sample 11 times in three different experiments and performed on different days. The sample was provided by Santiago de Compostela University Hospital Complex and the results of the study are listed in Table IV. The relative standard deviations (RSD) obtained were 3.2, 1.1, and 2.3%.

TABLE III
Aqueous Calibration Using Two Mercury Standards of 20 and 50 µg/L

Mercury Standard (µg/L)	Volume (µL)	Mercury Standard (ng)	µ Absorbance
Blank	0.300	0	6,599
20	0.125	2.5	36,541
20	0.250	5.0	62,532
20	0.375	7.5	86,776
20	0.500	10.0	119,258
50	0.300	15.0	162,878
50	0.400	20.0	219,320

Recovery of the Method

The analytical recovery was assessed for four concentration levels after spiking different aliquots of the blood sample with 5, 10, 15, and 20 µg L⁻¹ of Hg. The analytical recovery obtained gave a mean ± standard deviation of 103.2 ± 1.0%.

Accuracy of the Method

The accuracy of the method was studied with CRM Seronorm^{TM} Trace Elements Whole Blood, Level II, using aqueous calibration. The certified mercury concentration was 8.7 ± 1.3 µg/L and the found concentration obtained for five replicates was 9.86 µg/L. After statistical comparison (95% confidence level, student's *t*-test), no statistical difference between the certified mercury concentration and the found value was observed.

CONCLUSION

In this study, the direct determination of mercury in whole human blood using a mercury analyzer is discussed. The most important advantage of this technique is that the analysis of whole human blood can be performed directly in the liquid sample, avoiding sample digestion with wet digestion procedures. This reduces analysis time and possible sample contamination during pretreatment.

The detection limit obtained was 0.02 ng and the relative standard deviations were between 1.1% to 3.2%. Moreover, in this technique,

the use of oxidizing agents are avoided which is of benefit to Green Chemistry.

Since only small sample amounts are used, it is necessary to take precautions with regard to sample homogeneity and to perform at least three replicates. Due to the different behavior observed for the real samples, the CRM, and the aqueous standards, and in order to avoid possible problems with real samples, we recommend performing the calibrations with different amounts of a whole human blood certified reference material.

ACKNOWLEDGMENTS

We thank PerkinElmer España, S.L., Madrid, Spain, for lending the SMS 100 Mercury Analyzer instrument to perform this study.

Received March 9, 2011.

REFERENCES

1. T.W. Clarkson, Environ. Health Perspect 100, 31 (1993).
2. US EPA, EPA's Roadmap for Mercury, EA-HQ-OPPT-2005-0013 (2005), <http://www.epa.gov/mercury/roadmap/htm>.
3. F.M.M. Morel, A.M.L. Kraepiel, and M. Amyot, Ann. Rev. Ecol. Syst. 29, 543 (1998).
4. D.B. Porcella, Mercury in the environment: biogeochemistry, in C.J. Watras, J.W. Huckabee, Eds., Mercury Pollution Integration and Synthesis, Lewis Publishers, Boca Raton, FL, USA, pp. 3-19 (1994).

TABLE IV
Repeatability Study Analyzing a Real Blood Sample

Sample ID	Conc. (ng/g)	Conc. (ng/g)	Conc. (ng/g)
R1	9.0863	4.6744	10.7200
R2	8.6949	4.6881	10.0800
R3	8.8601	4.7679	10.3200
R4	8.9017	4.6606	10.2500
R5	9.0181	4.6972	10.4500
R6	9.3912	4.7310	10.4400
R7	8.9718	4.6582	10.3600
R8	9.4433	4.6313	10.5800
R9	9.3375	4.6002	10.2800
R10	9.5524	4.6192	9.7800
R11	9.5787	4.5893	10.3800
Mean	9.1669	4.6652	10.3309
SD	0.2904	0.0521	0.2371
RSD	3.2	1.1	2.3

5. T.W. Clarkson, Environ. Toxicol. Chem. 9, 957 (1990).
6. T.W. Clarkson, Environ. Health Perspect. 110, 11 (2002).
7. US EPA (2009). <http://www.epa.gov/waterscience/fish/advisories>
8. European Commission, Health and Consumers Directorate-General, Information Note, Subject: Methyl mercury in fish and fishery products, 21 April 2008, D/530286.
9. B.E. Erickson, Chemical & Engineering News, pp. 40-41, January 31 (2011).
10. US EPA Method 7473, <http://www.epa.gov/osw/hazard/tes/tmethods/sw846/pdfs/7473.pdf>
11. J. Cizdziel, T. Hinnert, and E. Heithmar, Water Air Soil Pollut. 135, 357 (2002).
12. C.T. Costley, K.F. Mossop, R.J. Dean, L.M. Garden, J. Marshall, and J. Carroll, Anal. Chim. Acta 405, 179 (2000).
13. J. Cizdziel and S. Gerstenberger, Talanta 64, 918 (2004).
14. B.S. Fox, K.J. Mason, and F.C. McElroy, J. ASTM Int. 2, 196 (2005).
15. The Determination of Total Mercury in Whole Blood Using Thermal Decomposition and Amalgamation Coupled with Atomic Absorption, PerkinElmer, Inc., Shelton, CT, USA (2009).
16. ASTM Method D6722-01. <http://www.atm.org/Standards/d6722.html>