

Determination of total mercury in whole blood by flow injection cold vapor atomic absorption spectrometry with room temperature digestion using tetramethylammonium hydroxide

Fernando Barbosa Jr.^{a,c} Christopher D. Palmer,^a Francisco J. Krug^c and Patrick J. Parsons^{*a,b}

^aWadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509, USA. E-mail: patrick.parsons@wadsworth.org

^bDepartment of Environmental Health and Toxicology, School of Public Health, The University at Albany, Albany, NY 12201-0509, USA

^cCentro de Energia Nuclear na Agricultura, Universidade de Sao Paulo, Caixa Postal 96, CEP 13400-970, Piracicaba-SP, Brazil

Received 9th January 2004, Accepted 30th April 2004

First published as an Advance Article on the web 12th July 2004

An improved method is described for the determination of total Hg in whole blood based on flow injection cold vapor atomic absorption spectrometry (FI-CVAAS). Aliquots of whole blood (300 μl) are diluted 1 + 1 v/v with 10% v/v tetramethylammonium hydroxide (TMAH) solution, incubated for 1 h at room temperature and then further diluted 1 + 4 v/v with 2% v/v HCl. The TMAH approach is directly compared to a previously published method that utilizes an on-line microwave-assisted heating step as part of the FI arrangement. Thus, the key issue addressed here is the elimination of the on-line microwave digestion step from the FI manifold, because of problems observed with precipitation in the reaction coil and high back pressure in FI manifold. Matrix-matched calibration standards are used and the method detection limit (3σ) is 0.2 $\mu\text{g l}^{-1}$. Method accuracy is traceable to Standard Reference Material (SRM) 966 Toxic Metals in Bovine Blood from the National Institute of Standards and Technology (NIST). Additional validation data are provided based on the analysis of whole blood materials from the New York State Department of Health's Proficiency Testing Program for Trace Elements and from the trace elements intercomparison program operated by the Institut National de Santé Publique du Québec, Canada. Accuracy (-2.5%) and precision ($<6.0\%$ repeatability and $<10\%$ intermediate precision) of the TMAH method compare favorably with values obtained for the on-line microwave method ($<10.0\%$ repeatability and $<15\%$ reproducibility), and the TMAH method is essentially free of the disadvantages associated with the latter method.

Introduction

Mercury (Hg) is a non-essential toxic element, and is known to affect the central nervous system in a number of ways.¹ The main sources of human exposure are the consumption of specific fish species that bioaccumulate Hg as a toxic organometallic compound, and the inhalation of Hg vapor released from dental amalgams. Methylmercury (MeHg) is the most common organomercury species found in seafood.¹

Measurements of Hg in both blood and urine are used to determine whether adverse health effects are likely to occur. Urinary Hg determination is more commonly used to assess exposure to inorganic forms of mercury (i-Hg). Measurements of Hg in whole blood, however, are used to assess exposure to organomercury compounds, such as MeHg, because the biological half-life of organomercury species in blood is significantly longer than it is for i-Hg compounds.² Methylmercury is rapidly absorbed from the gastrointestinal tract and accumulates in red blood cells. Organomercury compounds circulate in the blood for a long time, and gradually accumulate in the central nervous system, where their toxic effects are most pronounced. Normal concentrations of total whole blood Hg are typically less than 5.0 $\mu\text{g l}^{-1}$ in adults.² However, more recent data in the non-institutionalized US population suggest the 95th percentile for whole blood Hg is 2.30 $\mu\text{g l}^{-1}$ for young children and 7.10 $\mu\text{g l}^{-1}$ for adult females.³

One of the most widely used analytical techniques for the determination of Hg in clinical specimens is cold vapor atomic absorption spectrometry (CVAAS), which is typically

performed using dedicated analytical instrumentation.⁴⁻¹⁵ This well established technique is based on the amount of light absorbed at 253.7 nm by ground state atoms of Hg in the gas phase formed by reaction with a solution of NaBH_4 or SnCl_2 . A key step in the determination of total Hg in blood is to convert any organomercury species present into inorganic Hg^{2+} ions, which are then reduced to elemental Hg^0 . In the case of blood matrices, acid digestion both off-line and on-line, followed by CVAAS, has been proposed.^{4,7,9,13,14} An on-line sample pre-treatment method is preferable, because it reduces sample manipulation, analysis time, reagent consumption and risks of contamination.¹⁶ Guo and Baasner⁹ described an on-line digestion method for total Hg in whole blood based on flow injection (FI) coupled to CVAAS. Their method requires dilution of blood with a bromate-bromide reagent, followed by on-line sample decomposition in an HCl medium using microwave-assisted heating in a FI-CVAAS system. In the U.S., this method has been used successfully with minor modifications by the U.S. Centers for the Disease Control and Prevention (CDC) for total blood Hg measurements carried out as part of the National Health and Nutrition Examination Surveys (NHANES IV, 1998-2000).^{3,7} We have used essentially the same method and FI instrumentation as the CDC, with some minor modifications, for routine measurements of total Hg in whole blood in duplicate, with good accuracy and precision, at a rate of around 15 samples h^{-1} . However, some workers have reported difficulties with the FI instrumentation when blood specimens are analyzed, including blockages from precipitation of organic residues within the microwave reaction

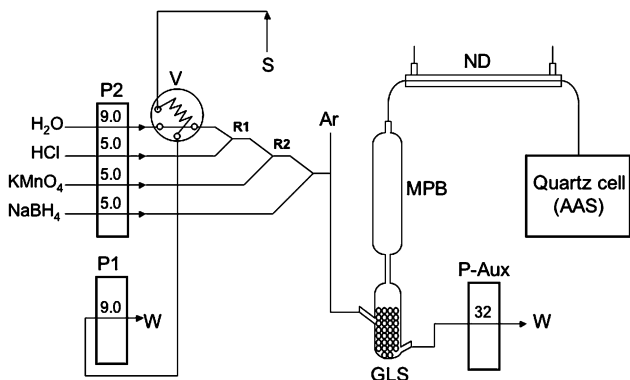


Fig. 1 Flow injection manifold for determination of total Hg in whole blood by CVAAS. Legend: V = FIMS 400 valve; R1, R2, = 10 cm, 0.8 mm id tubing; W = waste streams; MPB = modified pipet bulb; ND = PermaPure Nafion[®] Dryer; P-AUX = auxiliary waste pump.

coil, and troublesome back pressure within the FI manifold that may be the result of exothermic reactions occurring within the microwave cavity.¹⁷

Tetramethylammonium hydroxide (TMAH) has been previously used for sample pre-treatment as an alternative to microwave-assisted acid digestion of biological materials for the determination of Hg.^{18–24} For example, it was reported that various Hg species can be quantitatively extracted from fish tissues at room temperature using TMAH and an ultrasonic bath, and/or heating for a short period of time.²² Moreton and Delves²⁴ used TMAH in one of their diluents for the determination of total Hg in whole blood by ICP-MS. Here, we describe the feasibility of using TMAH to digest blood samples at room temperature, compared with microwave-assisted heating, before determination of total Hg by FI-CVAAS.

Experimental

Instrumentation

We used a PerkinElmer (P-E) Model 400 Flow Injection Mercury System (FIMS) equipped with an AS-90 autosampler for all Hg measurements (PerkinElmer Instruments, Shelton, CT). The FIMS 400 is a small, dedicated single-beam AAS instrument for Hg determinations that incorporates a low-pressure Hg lamp as a radiation source, and a photocell detector with a maximum sensitivity at the 253.7-nm resonance line for Hg. For the microwave-assisted method of Guo and Baasner,⁹ this instrument was coupled to an on-line Maxidigest MX 350 microwave digester (Prolabo, Paris, France) that can deliver a maximum output of 300 W. All system control and operations, including those for the Maxidigest MX 350 and AS-90, were accomplished using the P-E AA Winlab control software[™] version 2.50, running on a Pentium workstation. For the TMAH method, the microwave digester was not used. All analyses were carried out in duplicate and were based on peak height measurements because of the improvement in signal-to-noise close to the detection limit. Pure argon (99.998%) was used as the carrier gas.

Flow injection cold vapor generation

A schematic of the FI arrangement used for total Hg with TMAH digestion at room temperature is shown in Fig. 1. For comparison purposes the method of Guo and Baasner⁹ was used with minor modifications. Notably, in both TMAH and on-line microwave methods, a separate auxiliary peristaltic pump Minipuls 3 (Gilson, Inc., Middleton, WI) was used to handle waste removal from the gas-liquid separator and from the cooling lines from the microwave reaction coil. This modification eliminates problems associated with overloading the FIMS pump tubing magazine, permits better control of the waste-draining rate, and facilitates pump tubing replacement. As reported by others (Robert L. Jones, CDC, personal communication, 2002), we also found it preferable to use 10% v/v HCl rather than 5% v/v as had been reported by Guo and Baasner.⁹ We used matrix-matched calibration standards for both methods. The final FIMS parameters optimized for both methods are shown in Table 1.

We used 0.8-mm id PTFE[®] manifold tubing with the FIMS instrumentation and Tygon[®] peristaltic pump tubing with various diameters (Cole-Parmer, Vernon Hills, IL). Specifically, we used 1.14-mm id pump tubing (red-red) to propel the acid, KMnO₄, and NaBH₄ streams, 1.52-mm id pump tubing (yellow-blue) to propel the carrier and sample streams, and two 3.18-mm id pump tubes (black-white) that were connected to the waste drain of the gas-liquid separator by a T-piece. The gas-liquid separator (GLS) provided by P-E (part number B050-7959) for use with the FIMS 400 was found to be unsuitable for the analysis of diluted blood solutions, because of the tendency for moisture to enter the quartz absorption cell following a vigorous reaction between the blood matrix and the borohydride reagent. To minimize foaming in the FI manifold, a clear glass GLS (P-E part number B019-3772) filled with glass beads is used. This GLS is connected to the bulb of a modified 10-ml glass volumetric pipette positioned directly above so as to provide additional dead-volume, thus preventing foam from reaching the gas transfer line between the GLS and absorption cell. Although this modified GLS arrangement leads to some minor peak broadening, it does not affect the performance of the analysis. This GLS design was originally developed at the CDC and has been used for several years for their routine Hg determinations in blood (Robert L. Jones, CDC, personal communication, 2002). To minimize entrainment of water vapor into the quartz absorption cell, a Nafion[®] dryer tube (part number MD-070-12F2, Permapure, Toms River, NJ) was also connected to the outlet of the modified GLS arrangement. After each analytical run, a 50% v/v HCl solution was pumped through the system for 15 min, followed by deionized water for 10 min for system maintenance. In addition, the GLS and the FI manifold mixing tees were soaked overnight in 30% v/v HCl and were washed with deionized water before each analytical run. This procedure reduces residue formation inside the FI manifold; if ignored, such residue affects the Hg sensitivity over time.

Reagents and sample preparation

All reagents were of analytical-reagent grade unless otherwise specified. Double-deionized water with a final resistivity of

Table 1 FIMS-400 program for determination of total Hg in whole blood^a

Step No.	Time (s)	Pump 1 (rpm)	Pump 2 (rpm)	Valve position	Read
Prefill	5 (5)	90 (90)	120 (120)	Fill (Fill)	
1	8 (8)	90 (90)	120 (120)	Fill (Fill)	
2	20 (40)	90 (90)	0 (0)	Inject (Inject)	Read
3	1 (35)	90 (90)	0 (0)	Fill (Inject)	(Read)
4	0 (1)	0 (90)	0 (0)	Fill (Fill)	

^a Sample injection volume = 500 µl. (Values in parentheses refer to the on-line microwave digestion method. See text for details.)

18.2 MΩ cm was provided by a Milli-Q mixed bed ion-exchange system (Millipore, Bedford, MA). Ultrapure concentrated hydrochloric acid (Ultrax) was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). We used ACS-grade potassium permanganate from GFS Chemicals (Powell, OH), sodium tetrahydroborate (98% w/w) from Alfa Aesar (Ward Hill, MA), and ACS-grade sodium hydroxide from MCB reagents (Cincinnati, OH). A 25% w/w aqueous solution of tetramethylammonium hydroxide, electronic grade, 99.9999%, was obtained from Alfa Aesar. ACS grade K₂Cr₂O₇ (99.95–100.05% w/w) was purchased from Alfa Aesar.

A 0.5% m/v solution of K₂Cr₂O₇ prepared in 3% v/v HNO₃ was used as a stabilizer for Hg standards. A 0.2% m/v NaBH₄ solution in 0.05% m/v NaOH was prepared daily, and a 5-ml volume of 1 + 9 diluted antifoaming agent (Dow Corning 110 B) was added to 1 l of the NaBH₄ solution to minimize foam formation in the GLS. In addition, a 0.2% m/v solution of KMnO₄ was prepared daily in deionized water and was stored protected from light in a dark brown bottle, to minimize photodecomposition. A 10% v/v HCl solution was prepared by appropriate dilution of the concentrated acid; deionized water was used as a carrier in the FI system.

A 1000 mg l⁻¹ NIST-traceable standard solution of methylmercury chloride (MeHgCl) in water was obtained from Alfa Aesar. Serial dilution of this stock solution provided organomercury standards for checking recoveries. The MeHgCl standards are stored at 4 °C and are protected from light. Inorganic Hg calibration standards were prepared from a 1000 mg l⁻¹ NIST-traceable AAS Hg solution (The Aldrich Company, Milwaukee, WI). Five intermediate i-Hg standards were prepared by serial dilution of the stock solution, each containing 2% by volume of a K₂Cr₂O₇ stabilizing solution (0.5%, w/v) and 0.2% v/v HCl. Matrix-matched calibration standards were prepared daily by adding 300 μl of each intermediate Hg standard solution to five 15-ml Falcon tubes (Becton Dickinson Labware, Franklin Lakes, NJ), 500 μl of a base blood material, and 500 μl of 10% v/v TMAH. The calibration standards were incubated at room temperature for 1 h, and were diluted to a final volume of 5 ml with 2% v/v HCl such that the final base blood dilution was 1 + 9 (v/v). The base blood was obtained from a caprine (goat) source, and Hg concentrations were below the method detection limit of 0.2 μg l⁻¹.

Aliquots (300 μl) of human blood specimens, standard reference material (SRM) 966 Toxic Metals in Bovine Blood and various proficiency test (PT) samples were transferred to 15-ml Falcon tubes each containing 300 μl of 10% v/v TMAH solution. The micropipette used to aliquot the blood was “pumped” several times following dispensing of the specimen, to ensure thorough mixing and lysing of blood cells. Specimens and QC materials were incubated at room temperature for 1 h with TMAH. The TMAH-digested samples were diluted 1 + 4 with 2% v/v HCl to a final volume of 3 ml (a final sample dilution factor of 1 + 9). Before proceeding with the analysis, we vortexed diluted specimens, QC materials, and matrix-matched calibration standards for 2 min to ensure complete sample homogeneity.

Human blood specimens, proficiency testing samples, and certified reference materials

Standard Reference Material (SRM) 966 Toxic Metals in Bovine Blood was purchased from the National Institute of Standards and Technology (NIST), Gaithersburg, MD. Previously analyzed whole blood pools were obtained from (a) the New York State Department of Health's Trace Elements PT program and (b) the Institut National de Santé Publique du Québec, Canada, for additional validation purposes. Human blood specimens (*n* = 44) collected as part of another research study involving Hg exposure were available for method comparison purposes with identifiers removed. These specimens

had been obtained with informed consent from humans subjects specifically for t-Hg measurements in accordance with procedures approved by the New York State Department of Health's Institutional Review Board.

Results and discussion

Preliminary experiments were carried out to explore the feasibility of analyzing blood specimens by diluting them 1 + 9 with a 25% v/v TMAH solution directly. At this concentration of TMAH, a proteinaceous residue from the blood matrix is observed that adheres to the bottom of the tube, such that homogenization is very difficult. Therefore, we reduced the TMAH concentration to 10% v/v and coupled this with a 1 + 1 dilution of the blood specimen. This eliminated the proteinaceous residue problem noted above.

In an effort to assure complete recovery of Hg regardless of the endogenous species, *i.e.*, i-Hg or MeHg, two aliquots of base blood were diluted 1 + 9 with a 10% v/v TMAH solution, spiked with respectively (i) 5 μg l⁻¹ i-Hg and (ii) 5 μg l⁻¹ MeHg, and incubated at room temperature for 2 h. These spiked materials were analyzed using the FI arrangement shown in Fig. 1, *i.e.*, with KMnO₄ (0.05% m/v), NaBH₄ and 10% v/v HCl. A significant decrease (~60%) in peak height sensitivity for both i-Hg and MeHg was observed, compared with the on-line microwave decomposition method.⁹ The observed decrease in sensitivity may be due to the lower extraction efficiency of TMAH for Hg in a blood matrix, or it may result from an excess of hydroxide ions produced after TMAH digestion is complete—the pH of the latter is approximately 12 after the alkaline digestion. Such alkaline conditions may not be favorable for Hg reduction by NaBH₄. To explore this hypothesis further, we repeated TMAH digestion using higher concentrations of HCl (up to 60% v/v) in the FI system. Fig. 2 shows the improvement in peak height sensitivity for Hg as the HCl concentration is increased, reaching a plateau for acid concentrations above 3.6 mol l⁻¹ (30% v/v).

However, the use of HCl concentrations above 10% v/v causes protein precipitation within the GLS. To avoid this, blood specimens must be diluted in two steps. They are first diluted 1 + 1 with 10% v/v TMAH, as noted above, incubated for 2 h and then diluted by a further 1 + 4 with 2% v/v HCl. The selection of 2% v/v HCl was based on optimization studies

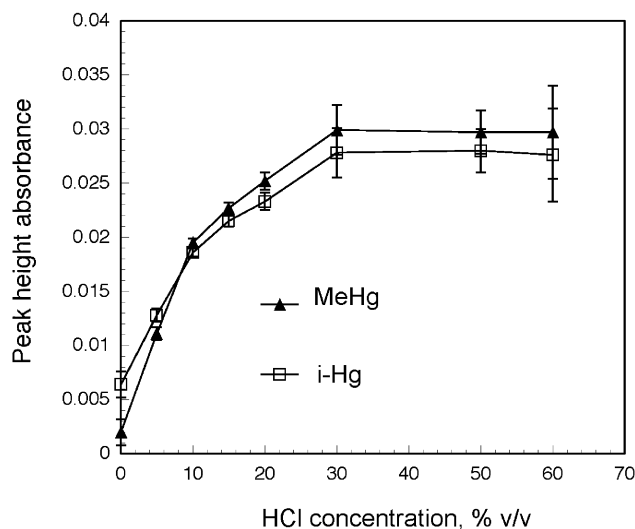


Fig. 2 Effect of hydrochloric acid concentration on Hg sensitivity following blood sample digestion with TMAH. Data shown include 5 μg l⁻¹ MeHg (▲) and 5 μg l⁻¹ i-Hg (□) spiked into a base blood pool, and diluted 1 + 9. Error bars indicate the range for triplicate measurements.

showing the absence of protein precipitation. All subsequent work utilized this dilution protocol.

KMnO₄ and NaBH₄ concentrations

Potassium permanganate is frequently used in FI-CVAAS methods to oxidize those organomercury species present to inorganic Hg²⁺. When this approach is combined with a subsequent on-line reaction using sodium borohydride, most organomercury species found in biological specimens are subsequently reduced and are easily determined by CVAAS, as reported by Guo and Baasner for blood⁹ and urine,⁶ and by Tao *et al.*¹⁸ for fish tissues. One problem that has been reported with use of KMnO₄ solutions in FI-CVAAS is the formation of a film of hydrated manganese(IV) oxide on the surface of the manifold components.¹⁷ Hanna and McIntosh¹⁷ used hydroxylamine hydrochloride to dissolve this oxide film. We also encountered this problem, although we found that the film formation could be avoided by reducing the residence time of the reaction mixture in the FI system, or by using a higher concentration of NaBH₄ relative to KMnO₄. We investigated the latter by altering the concentration of KMnO₄ relative to NaBH₄. Two aliquots of base blood were diluted 1 + 1 with 10% v/v TMAH and were incubated for 2 h as described previously, followed by a 1 + 4 dilution with 2% v/v HCl. The diluted blood was spiked with Hg to produce (i) 4 μg l⁻¹ i-Hg and (ii) 4 μg l⁻¹ MeHg. The concentration of KMnO₄ and NaBH₄ was varied from 0.05% to 0.3% m/v, but an equal or greater concentration of NaBH₄ relative to KMnO₄ was always maintained. Under these conditions, film residues of hydrated manganese(IV) oxide were not observed. Optimized conditions for spiked i-Hg and MeHg blood materials based on peak height absorbance were found to be 0.2% m/v KMnO₄ and 0.2% m/v NaBH₄. At these concentrations, i-Hg and MeHg had identical sensitivities based on peak height. Our observations are consistent with those reported previously by Tao *et al.* for t-Hg determination in TMAH-solubilized fish tissues.¹⁸ The optimized concentrations of KMnO₄ and NaBH₄ used here are typically higher than those used when blood is digested on-line using microwave-assisted heating. Therefore, in the absence of microwave heating, much stronger oxidizing conditions are necessary to achieve complete recovery of Hg from blood matrices. No attempt was made to recover organomercury species other than MeHg, since this is considered one of the more difficult species to reduce, and it is the predominant species found in human blood.¹ However, we recognize the possibility that there may be some obscure organomercury species that require different digestion conditions.

Carrier gas flow rate

The effect of Ar carrier gas flow rate on Hg sensitivity was investigated as a function of peak height absorbance. Gas flow rates were varied from 40 to 200 ml min⁻¹, based on the conditions shown in Table 2. Two base-blood materials were

Table 2 Optimized parameters for determination of total Hg in blood by flow injection cold vapor atomic absorption spectrometry

Instrumental parameter	Setting
Wavelength/nm	253.7
Measurement mode	Peak height
Read time/s	30
Read delay/s	0
BOC time/s	5
Microwave power	20%
Cold vapor flow injection parameter	
NaBH ₄ concentration (% m/v)	0.2
HCl concentration (% v/v)	10
Argon flow rate/ml min ⁻¹	75
Sample loop/μl	500
KMnO ₄ concentration (% m/v)	0.2

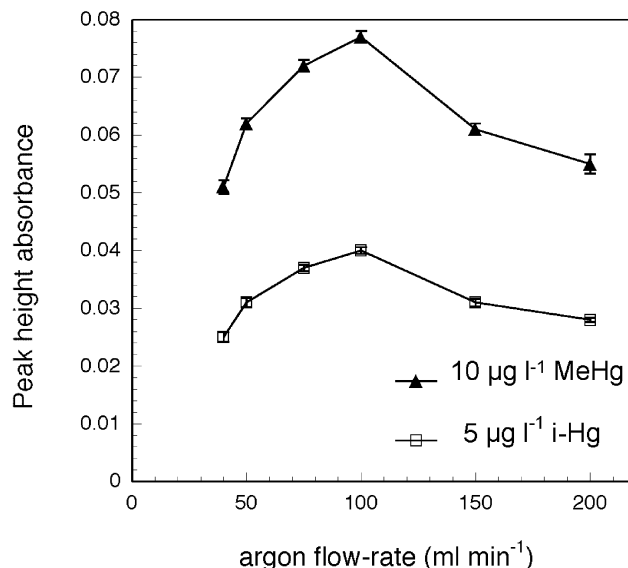


Fig. 3 Effect of argon gas flow rate on Hg sensitivity following TMAH digestion. Data shown include 10 μg l⁻¹ MeHg (▲) and 5 μg l⁻¹ i-Hg (□) spiked into a base blood pool, and diluted 1+9. Error bars are the range for triplicate measurements.

treated with 10% v/v TMAH and spiked with Hg to produce (i) 5 μg l⁻¹ i-Hg, and (ii) 10 μg l⁻¹ MeHg and were used to optimize Ar flow rate. Fig. 3 shows the effect on peak height absorbance as the flow rate is increased to 200 ml min⁻¹. At gas flow rates above 75 ml min⁻¹, the evolution of foam in the gas-liquid separator becomes more troublesome, affecting Hg results. Therefore, 75 ml min⁻¹ was selected as the optimal Ar carrier gas flow rate.

Incubation time

Incubation time was optimized using NIST SRM 966 Toxic Metals in Bovine Blood. In each of five tubes, 300 μl of SRM 966 was diluted 1 + 1 with 10% v/v TMAH; the tubes were incubated at room temperature for 30, 60, 120, 180 or 360 min. Following incubation, the blood was then diluted 1 + 4 with 2% HCl v/v, for a total dilution factor of 1 + 9. Optimized analytical conditions are shown in Table 2. After a 1-h incubation with TMAH at room temperature, good recoveries were found based on the certified Hg value of 31.4 μg l⁻¹. Therefore, 1 h was selected as the optimal incubation time for blood.

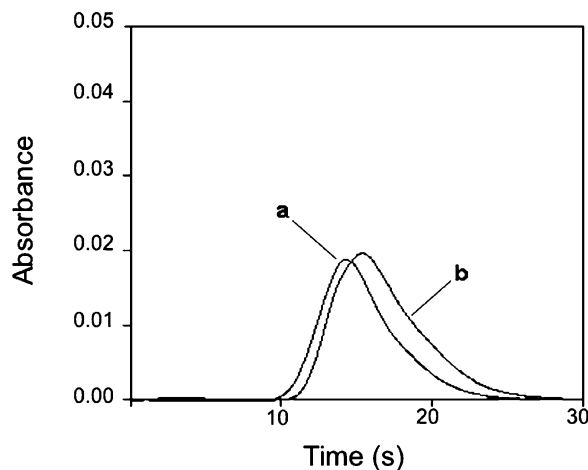


Fig. 4 Typical Hg FI absorption profiles observed following TMAH digestion: a, 1 + 9 v/v diluted base blood sample spiked with 3.0 μg l⁻¹ Hg, and b, 1 + 9 v/v diluted NIST SRM 966 bovine blood (level two certified value: 31.4 ± 1.7 μg l⁻¹ Hg).

Typical Hg absorption profiles are shown in Fig. 4 for (a) a base blood material spiked with $30 \mu\text{g l}^{-1}$ i-Hg and (b) NIST SRM 966 Toxic Metals in Bovine Blood level 2; both blood samples were diluted 1 + 1 with 10% TMAH and incubated for 1 h before a 1 + 4 dilution with 2% v/v HCl. Optimized analytical conditions are shown in Table 2.

Validation studies

Validation of the TMAH method was accomplished using NIST SRM 966 Bovine Blood as well various secondary reference materials, either provided by the New York State Department of Health's PT program for trace elements in whole blood, or obtained from the external quality assessment scheme (EQAS) for trace elements operated by the Institut National de Santé Publique du Québec (INSP), Canada. For the purpose of additional validation, we analyzed 44 human blood specimens using the TMAH method and compared these data to results obtained using the on-line microwave-assisted sample digestion method.⁹ Matrix-matched calibration standards were used for both methods.

Results for NIST SRM 966 and the PT/EQAS samples are shown in Table 3. Intermediate precision reported for PT/EQAS results is based on duplicate injections performed over a 4-day period for the TMAH method and duplicate injections over a 3-day period for the microwave-assisted heating method. Values found using the TMAH method are in good agreement with established target values. For method comparison studies involving human blood specimens, it is desirable to view the data both as a scatter plot and as a difference plot. When two methods of equal imprecision are compared, a Bland-Altman (B-A) difference plot is recommended.^{25,26} Fig. 5 shows a comparison between the TMAH and on-line microwave-assisted digestion methods for total Hg determination in 44 human blood specimens as (a) a scatter plot and (b) a B-A difference plot. Examination of the B-A plot in Fig. 5b shows that only nine data points out of the 44 exceeded $5 \mu\text{g l}^{-1}$; of those, only four were above $10 \mu\text{g l}^{-1}$, thus rendering inferences about bias estimates above $5 \mu\text{g l}^{-1}$ tenuous at best. To ensure the absence of bias, it is prudent to examine data provided by standard reference materials and samples from PT/EQAS programs at elevated Hg concentrations. The data from Fig. 5 and from Table 3, when taken together, do not provide any strong evidence of a bias one way or the other.

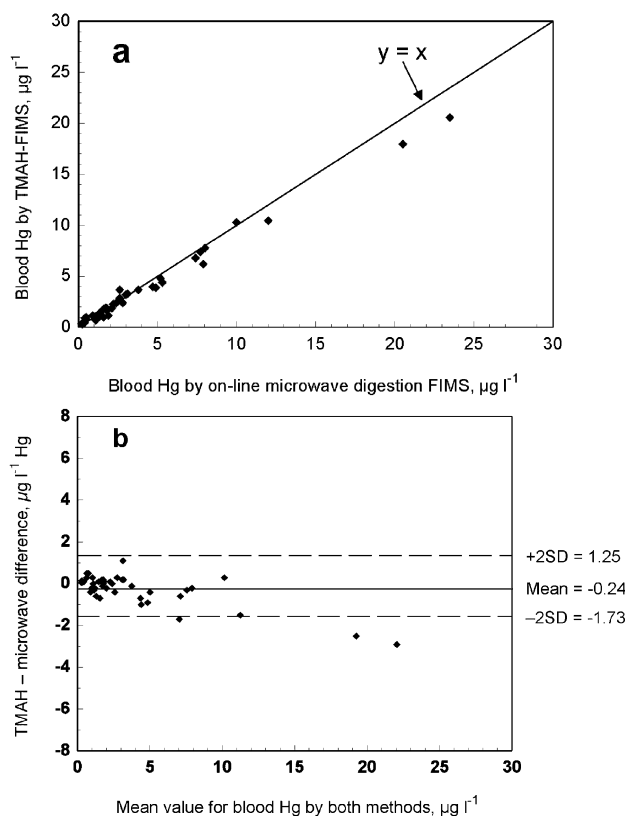


Fig. 5 Comparison between off-line TMAH digestion and on-line microwave digestion, for determination of total Hg in 44 human blood specimens (mean $3.8 \mu\text{g l}^{-1}$, range $0.2\text{--}23.5 \mu\text{g l}^{-1}$) by FI-CVAAS. Data are shown as: a, a scatterplot graphic; and b, as a Bland-Altman difference plot with $\pm 2\text{SD}$ limits.

Analytical characteristics

The TMAH method detection limit (3 SD) is $0.2 \mu\text{g l}^{-1}$, based on the analysis of 1 + 9 diluted base blood ($n = 10$), and the linear dynamic range extends from the MDL to $50 \mu\text{g l}^{-1}$ Hg. Typical within-day precision was $<6\%$, while between-day precision was $<10\%$ RSD. Typical sample throughput is limited to 38 h^{-1} , based on duplicate measurements. Comparison of figures-of-merit between the TMAH method and various published methods for Hg determination in whole blood by CVAAS are shown in Table 4. Mercury losses were

Table 3 Analytical performance for the determination of total Hg in whole blood by FI-CVAAS: comparison between on-line microwave digestion and room temperature digestion with TMAH

Sample	Target value/ $\mu\text{g l}^{-1}$ Hg	CVAAS with microwave digestion/ $\mu\text{g l}^{-1}$ Hg	CVAAS with TMAH digestion/ $\mu\text{g l}^{-1}$ Hg
NIST SRM 966	31.4 (1.7) ^a	30.6 (1.7) ^d	30.2 (1.5) ^d
NYS TE02-01	5.2 (2.2–8.2) ^c	5.4 (0.6) ^b	5.4 (0.4) ^b
NYS TE02-02	1.2 (0.0–4.2) ^c	1.0 (0.2) ^b	0.9 (0.2) ^b
NYS TE02-03	17.8 (14.2–21.4) ^c	17.8 (1.7) ^b	19.1 (0.3) ^b
NYS TE02-04	33.9 (27.1–40.7) ^c	34.6 (3.5) ^b	35.9 (2.2) ^b
NYS TE02-05	7.3 (4.0–10.3) ^c	6.7 (0.6) ^b	6.8 (0.6) ^b
Quebec ICP00B-01	11.00 (9.3–12.7) ^f	10.7 (1.2) ^c	9.3 (0.1) ^c
Quebec ICP00B-02	3.1 (1.75–4.45) ^f	3.6 (0.2) ^c	3.1 (0.4) ^c
Quebec ICP00B-03	9.18 (7.5–10.9) ^f	10.0 (0.9) ^c	8.9 (0.5) ^c
Quebec ICP01B-02	18.1 (14.3–21.9) ^f	18.7 (1.5) ^c	18.8 (3.0) ^c

^a NIST Standard Reference Material 966 Bovine Blood, spiked with inorganic and organic mercury. Certified values established using data for total mercury obtained by two different techniques: electrothermal atomic absorption spectrometry (ETAAS) and flow injection atomic absorption spectrometry (FIAAS) at the Centers for the Disease Control and Prevention (CDC), Atlanta, GA. Uncertainty is defined as the expanded uncertainty at the 95 % level of confidence (Student's *t*-test). ^b Analyzed in duplicate over 4 days (\pm SD). ^c Analyzed in duplicate over 3 days (\pm SD). ^d Analyzed in triplicate over 3 days (\pm SD). ^e NYS Trace Elements in whole blood PT materials: target values are the mean of 11 referee laboratories using either quadrupole-based ICP-MS instrumentation and/or a cold vapor AAS method. Acceptable ranges are fixed at $\pm 20\%$, or $\pm 3 \mu\text{g l}^{-1}$ around the target value, whichever is greater. ^f INSP-Quebec ICP-MS Comparison program; target values are the participant mean excluding outliers. Acceptable range is based on z-score statistics.

Table 4 Analytical performance parameters for determination of total Hg in whole blood by FI-CVAAS: comparison between published methods and the TMAH digestion method

Analytical parameter	This method (TMAH)	Guo and Baasner ⁹ (1993) ^a	Dressler <i>et al.</i> ¹³ (2002) ^b
Method detection limit/ng l ⁻¹	200	170	20
Sample throughput/h ⁻¹	38	15	12
Characteristic mass/pg	314	261	Not reported
Calibration	Matrix-matched	Matrix-matched	Aqueous
Sample digestion	Pre-treatment with TMAH	On-line microwave	Off-line microwave
Repeatability (%) (within day) (<i>n</i> = 4) (NYS TE02-01) ^c	<6.0	<10.0	Not reported
Intermediate precision (%) (between day) (<i>n</i> = 4) (NYS TE02-01) ^c	<10.0	<15.0	Not reported

^a Based on data obtained in our laboratory (for method details see ref. 9). ^b Based on published data.¹³ ^c Target value = 5.2 µg l⁻¹ Hg.

not observed even after 24 h of blood sample preparation combined with storage at 4 °C, showing that blood samples can be prepared one day and analyzed the next.

Conclusions

A simple method for preparing blood samples for total Hg determination using on-line FI-CVAAS instrumentation was developed, based on room temperature digestion with TMAH. This approach negates the need for on-line microwave-assisted heating to handle organomercury species in blood, thus avoiding several practical difficulties. Typical sample throughput is approximately 38 h⁻¹ based on duplicate analysis, and the TMAH method detection limit of 0.2 µg l⁻¹ is comparable to that reported for on-line microwave digestion methods.^{7,9}

Acknowledgements

The authors thank Dr. Robert L. Jones, Dr. Kathleen Caldwell and Mr. Ron Albalak of the CDC Inorganic Toxicology Laboratory for helpful advice and recommendations. Dr. F. Barbosa Jr. is grateful to Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP), processo 99/07645-8 for support associated with his visiting position at the Wadsworth Center.

References

- 1 *Toxicological Profile for Mercury (update)*, Agency for Toxic Substances and Disease Registry (ATSDR), U.S. Department of Health and Human Services, Atlanta, GA, 1999, p. 617.
- 2 *Control of Pre-Analytical Variation in Trace Element Determinations; Approved Guideline*, National Committee for Clinical Laboratory Standards (NCCLS) Document C38-A, 1997, 15.
- 3 *Second National Report on Human Exposure to Environmental Chemicals*, NCEH Pub. No. 02-0716, Centers for Disease Control, Atlanta, GA, 2003, pp. 1–251.
- 4 D. E. Nixon, G. V. Mussmann and T. P. Moyer, *J. Anal. Toxicol.*, 1996, **20**, 17–22.
- 5 C. P. Hanna, J. F. Tyson and S. McIntosh, *Anal. Chem.*, 1993, **65**, 653–656.
- 6 T. Guo and J. Baasner, *Anal. Chim. Acta*, 1993, **278**, 189–196.
- 7 H. P. Chen, D. C. Paschal, D. T. Miller and J. C. Morrow, *At. Spectrosc.*, 1998, **19**, 176–179.
- 8 M. Gallignani, H. Bahsas, M. R. Brunetto, M. Burguera, J. L. Burguera and Y. P. de Pena, *Anal. Chim. Acta*, 1998, **369**, 57–67.
- 9 T. Guo and J. Baasner, *Talanta*, 1993, **40**, 1927–1936.
- 10 M. R. Brunetto, J. R. Luna, A. Zambrano, M. Gallignani, M. Burguera, J. L. Burguera and Y. P. de Pena, *Analyst*, 1999, **124**, 1493–1499.
- 11 J. L. Lopez-Colon, D. Veiga, A. Montel, J. M. De Pradena and R. Lozano, *At. Spectrosc.*, 2002, **22**, 284–289.
- 12 B. Welz, D. L. Tsalev and M. Sperling, *Anal. Chim. Acta*, 1992, **261**, 91–103.
- 13 V. L. Dressler, E. M. Flores, D. Pozebon and L. E. Kaercher, *J. Anal. At. Spectrom.*, 2002, **17**, 790–793.
- 14 I. A. Bergdahl, A. Schuetz and G. A. Hansson, *Analyst*, 1995, **120**, 1205–1209.
- 15 J. E. Tahan, V. A. Granadillo, J. M. Sanchez, H. S. Cubillan and R. A. Romero, *J. Anal. At. Spectrom.*, 1993, **8**, 1005–1010.
- 16 M. Burguera and J. L. Burguera, *Anal. Chim. Acta*, 1998, **366**, 63–80.
- 17 C. P. Hanna and S. A. McIntosh, *At. Spectrosc.*, 1995, **16**, 106–114.
- 18 G. H. Tao, S. N. Willie and R. E. Sturgeon, *Analyst*, 1998, **123**, 1215–1218.
- 19 R. G. L. Silva, S. N. Willie, R. E. Sturgeon, R. E. Stanelli and S. M. Sella, *Analyst*, 1999, **124**, 1843–1846.
- 20 D. Pozebon, V. L. Dressler and A. J. Curtius, *J. Anal. At. Spectrom.*, 1998, **13**, 1101–1105.
- 21 D. Pozebon, V. L. Dressler and A. J. Curtius, *Talanta*, 2002, **51**, 903–911.
- 22 C. M. Tseng, A. De Diego, F. M. Martin, D. Amouroux and O. F. X. Donard, *J. Anal. At. Spectrom.*, 1997, **12**, 743–750.
- 23 G. H. Tao, S. N. Willie and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 1999, **14**, 1929–1931.
- 24 J. A. Moreton and H. T. Delves, *J. Anal. At. Spectrom.*, 1998, **13**, 659–665.
- 25 J. M. Bland and D. G. Altman, *Lancet*, 1986, **1**, 307–310.
- 26 J. M. Bland and D. G. Altman, *Lancet*, 1995, **346**, 1085–1087.