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Determination of antimony in human blood with inductively coupled plasma-mass spectrometry†

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A method is presented for the determination of antimony in whole human blood samples with an ICP-MS instrument using a quadrupole mass analyzer. A nitric acid/hydrogen peroxide open digestion procedure was employed for the blood sample treatment and preparation for analysis. The precision and accuracy of the method were evaluated by analyzing several Seronorm™ trace elements whole blood reference materials. The precision of the method at various antimony levels was better than 4% RSD and the recovery was greater than 92% at all levels. The detection limit, calculated as three times the standard deviation of the blank (3σ, $n = 12$), was 0.03 μg L^{-1}. The method was successfully applied for the determination of antimony in blood samples from school children in rural areas of Kwazulu/Natal, South Africa and adults from Dearborn, Michigan. Blood antimony levels ranged from <0.03 to 3.82 μg L^{-1} in children and 1.40 to 4.35 μg L^{-1} for adults.

Introduction

Contamination of the environment with antimony is of growing concern. Antimony is a potentially toxic trace element that has no known biological function.[1] Antimony trioxide is used as a flame retardant in various products, and antimony trisulfide is used as a lubricant in brake pads[2,3] and is also present in certain cosmetic and grooming products.[4,5] Antimony is used in producing and improving many industrial and commercial materials such as various alloys, ceramics, glasses, plastic, and synthetic fabrics.[1] In addition to dissipative uses, large quantities of antimony are released to the atmosphere from incinerators, smelters, combustion of fossil fuels, burning of garbage in the open air (especially in the developing countries) and industrial thermal processes that employ this element.[6,7] As a result of outputs from anthropogenic sources, much higher levels of antimony are measured in urban areas and around industrial point sources than would be expected from its natural occurrence.[8–10]

Environmental exposure to antimony has become a matter of some public health interest. Several analytical techniques for biomonitoring antimony in human and animal blood and other biological samples have been reported, and methodologies for the determination of total antimony in terrestrial environmental samples have been recently reviewed.[11] Neutron activation analysis has been used with reported detection limits of 5 μg L^{-1} in human blood and 1.1 μg L^{-1} in animal blood, respectively.[12,13] Antimony(III) and antimony(V) determination in liver tissues and whole blood by flow injection-hydride generation atomic absorption spectroscopy have been reported with detection limits of 1.0 μg L^{-1} for antimony(III) and 0.5 μg L^{-1} for antimony(V).[14] Anodic stripping voltammetry and graphite furnace atomic absorption spectrometry have been applied to the determination of antimony in biological matrices with reported detection limits of 20 and 25 μg L^{-1}.[15] Methods have been developed and applied for the determination, speciation and biomonitoring of antimony in the urine of non-exposed and exposed subjects.[16–18] Inductively coupled plasma-mass spectrometry (ICP-MS) with online ion chromatography has been used to monitor antimony species in biological samples from patients treated for leishmaniasis with reported detection limits of 1.6 μg L^{-1}.[19] Nitric acid-microwave digestion and high resolution ICP-MS for multielement analysis of calf blood, freeze-dried animal blood and human blood have been used for antimony analysis.[20,21] In one of these studies,

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antimony in blood collected from non-smoking healthy athletes was determined with a method detection limit of 0.022 μg L^{-1}.[20] Analysis of antimony in infant blood with ICP-MS has been reported.[22] Recently, a multi-elementary metal and metalloid method with ICP-MS has been used for the determination of antimony and other elements in biological fluid.[23]

Despite various medicinal and industrial applications and possible health risks from antimony compounds, the available information on the determination of antimony in human blood is very limited, especially for children. Some of the reported ICP-MS methods lack validation with reference blood samples. Additionally, the analysis of undigested whole blood was done after dilution where the high content of organic compounds may cause problems with the nebulizer and carbon deposition on the sampling orifice.[24] Even with segmented-flow technique, denatured proteins build up necessitating daily cleaning of the torch.[25] Furthermore, precautionary measures for handling undigested blood must be taken during sample preparation and instrumental service routines.[20] In this paper, we report on the determination of antimony in whole blood samples for school children and adults using nitric acid–hydrogen peroxide digestion and a quadrupole mass analyzer ICP-MS. Antimony concentrations were determined in blood samples collected from 49 South African children in six different schools located in rural areas of Kwazulu/Natal, South Africa and from nine Middle Eastern men living in the metropolitan Detroit area, Michigan, USA. The method was validated by analyzing three Seronorm™ trace elements whole blood reference materials containing different levels of antimony. The method reported has a better detection limit and suffers from less matrix effects and other metal interference compared to neutron activation analysis, anodic stripping voltammetry, graphite furnace atomic absorption spectrometry, and flow injection-hydride generation atomic absorption spectrometry.

Experimental

Sample collection and analysis

One group of blood samples used in this study was obtained from Grade 1 pupils (8–10 years old) from Vulamehlo, a rural area located about 90–140 km from Durban, Kwazulu/Natal, South African. The following elementary schools participated in the study: Dududu, Hluthankungu, Vukaphi, KwaMaquza, Nomandla, Dumisa, Soul, St. Michaels, Tholimfundo, and Zamafuthi. About 2.5 ml of the venous blood was drawn, by a trained nurse, into a lead-free plastic vacuutainer containing dry potassium EDTA. The puncture point was cleansed with alcohol, soapy water and then with distilled water. The blood was drawn, then carefully mixed with the anti-coagulant, and stored in a cooler box. All samples were frozen as soon as they got back to the laboratory. The blood samples were collected in 1995. Details of the sample collection and handling procedures are provided in Nriagu *et al.*[26]

The other set of blood samples came from adults who attended a community clinic in Dearborn, Michigan, USA. The cleaning of puncture point and draw of blood followed basically the procedure outlined above for the children. The samples were collected in 2005.

Reagents

MilliQ water, high purity nitric acid (Optima, Fisher Scientific, Canada) and hydrogen peroxide 30% Supra-pur® (MERCK KGaA, Darmstadt, Germany) were used in the sample treatment and preparation.

The polyethylene bottles used for storing the digested blood samples, plastic pipette tips, polypropylene volumetric flasks, and Teflon® tubes and bottles were cleaned and decontaminated using an established nine step procedure.[27] Between analysis, the Teflon® tubes were rinsed with acetone to remove a ring that formed on some of them and filled with 8 M nitric acid, triple bagged, and left at 45 °C for 48 h. The flasks and bottles were filled with 2 M trace metal nitric acid, triple bagged, and left at 45 °C for 48 h in a water bath. Then the Teflon® tubes, flasks, and bottles were drained, rinsed, filled with 0.2% (V/V) trace metal nitric acid, and triple bagged for storage. Rainin and/or Eppendorf electronic pipettes as well as spinal syringes were used to dispense the various blood samples. A Fisher Isotemp 145D dry-bath incubator was used for sample digestion.

Agilent Environmental Calibration standard P/N 5183-4688 (10 μg mL^{-1} antimony) was used to prepare working standard solutions. Spex CertiPrep single element ICPMS standard (1000 mg L^{-1} indium) was diluted and used as the internal standard. The reference materials used were Seronorm™ trace elements whole blood at three different antimony levels.

Sample digestion

Aliquots of 1.00 mL from Seronorm™ trace elements whole blood reference material, human blood, and MilliQ water (to serve as blank) were each dispensed into a pre-weighed Teflon® tube. The Teflon® tubes were placed in heating blocks of a dry-bath incubator which was set at 25 °C. An aliquot of 2.00 mL of Optima Fisher concentrated nitric acid was added to each Teflon® tube. The dry-bath incubator was heated stepwise to 70 °C, 85 °C, and 95 °C. The samples were maintained at 95 °C until the blood samples turned clear light brown. Some of the human blood samples displayed a greenish tinge as they were being digested; however, this changed to clear light brown at the end of the digestion. The dry-bath incubator was cooled to 35 °C. Next, 2.00 mL of hydrogen peroxide 30% Supra-pur® was added to each tube. The temperature was raised to 60 °C and the tubes were heated for 10 min. The temperature was then raised to 65 °C, and maintained for 10 min. The temperature was increased to 70 °C, and heating was continued for 20 min. The temperature was then elevated to 75 °C, followed by the addition of 1.00 mL of hydrogen peroxide 30% Supra-Pur® to each tube. The samples were heated for 10 min. This was followed by raising the temperature to 80 °C and maintaining this level for 30 min. Finally, the temperature was increased to 95 °C and the

Table 1 Agilent 7500c quadrupole ICP-MS operating conditions

RF power:	1600 W
Reflected power:	<5 W
Sample uptake rate:	0.4 mL min^{-1}
Gas flow rates:	Plasma gas: 15 L min^{-1} Auxiliary gas: 0 L min^{-1} Carrier gas: 0.86 L min^{-1} Makeup gas: 0.29 L min^{-1}
Ion sampling depth:	8 mm
Torch:	Fassel, Quartz 2.5 mm id fitted with Agilent ShieldTorch system
Nebulizer:	Glass expansion micromist (0.4 mL min^{-1} uptake)
Cone:	Platinum, orifice diameter 1.0 mm
Skimmer:	Platinum, orifice diameter 0.4 mm
Spray chamber:	Water cooled Scott type (Double Pass) (2 °C)
Acquisition:	Full quantitative mode with no reaction gas (*i.e.* normal mode) Antimony: 3 points $peak^{-1}$; 1.0 s $point^{-1}$ with three replicates Internal standard (~10 μg L^{-1} added online using T-connector) Indium (115,118): 3 points $peak^{-1}$; 0.1 s $point^{-1}$ with three replicates Washout to <1% carryover, rinse with 5% nitric acid

Table 2 Validation of the method using Seronorm™ trace elements whole blood reference materials ($n = 5$ for each level) and human whole blood for antimony

Level, lot number	Amount claimed/μg L^{-1}	Uncertainty/μg L^{-1}	Amount found/μg L^{-1}	% RSD	% Recovery
1, OK336	1.64	1.52–1.76	1.51	3.8	92.1 ± 3.5
2, OKMR9067	26	25–28	27.1	2.1	104.3 ± 2.2
3, OK0337	83	77–89	82.1	2.9	98.9 ± 2.9
Human whole blood			1.78	2.2	

samples were heated for 30 min or until the gas bubbling stops. Heating in a stepwise fashion was necessary after the addition of hydrogen peroxide because it decomposes at elevated temperatures.[28] Additionally, this reduced excessive effervescence and subsequent loss of sample.

The samples were diluted to 25 mL using polypropylene volumetric flasks, and transferred to clean polyethylene bottles for storage prior to ICP-MS analysis.

Instrumental analysis

An Agilent 7500c quadrupole ICP-MS equipped with a dynamic reaction cell and a Cetac ASX-500 autosampler was used to measure the concentration of antimony in the digested samples. Instrumental operating conditions are given in Table 1.

Results and discussion

Calibration graph

A calibration graph ranging from 0 to 25 μg L^{-1} of antimony in 5% Optima Fisher concentrated nitric acid solution was prepared. The internal standard, 10 μg L^{-1} of indium, was added to each online using a T-connector.

Detection limit

The method detection limit was calculated as three times the standard deviation of the blank obtained by analyzing twelve 1.00 mL aliquots of MilliQ water following the described analytical procedure. The value was found to be 0.03 μg L^{-1}.

Accuracy and precision

To validate the method and to ascertain its precision and accuracy, five 1.00 mL aliquots of Seronorm™ trace elements whole blood reference materials at three antimony levels were analyzed following the described analytical procedure. In addition, five 1.00 mL aliquots of a human whole blood sample were similarly analyzed in quintuplate. The results are presented in Table 2. Good agreement between the claimed and found amounts was obtained at all levels and the % RSD was less than 4% for all determinations.

Interference

Antimony was measured at its m/z ratio of 121 using In (m/z = 115) as internal standard. For the digested blood sample matrix analyzed instrumentally, the spectral interferences on these masses were negligible and no corrections were necessary.

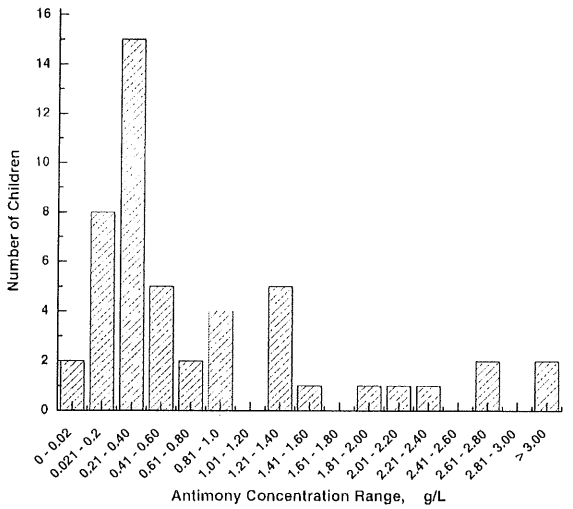

Fig. 1 Antimony concentrations in whole blood samples from South African school children.

Concentrations of antimony in blood samples

The statistical summary of the results of the analysis of the human whole blood samples from 49 South African school children and from nine Middle Eastern men living in the metropolitan Detroit area, Michigan, USA, are presented in Table 3. For the South African school children, only two samples (4.1%) were at or below the detection limit and 16.3% were in the range of 0.04–0.20 μg L^{-1}. The majority of the samples, 53.1%, were in the range of 0.21–1.0 μg L^{-1}, and 26.5% were between 1.21–3.82 μg L^{-1}, as illustrated in Fig. 1. The values and spread of results found in the rural community suggests that blood may be a valid biomarker of antimony exposure, although the likely sources of the antimony in the children's environment were not explored in this study. A study of leishmaniasis patients treated with *N*-methyl meglumine antimoniate showed that after exposure, antimony in blood decreases slowly over several months,[19] implying that blood antimony (SbB) levels can be a meaningful measure of past exposures.

Few previous measurements on blood Sb levels in children have been reported. Delves *et al.*[22] reported an average SbB value of 0.26 μg L^{-1} (range: 0.18–0.88 μg L^{-1}) for a random sample of 15 Irish infants less than one year old. Their samples were analyzed using an ICP-MS method. The mean of the SbB levels in South African school children (0.85 μg L^{-1}) is three times higher than that reported by Delves *et al.*[22] and the difference may be related to the fact that samples were not digested and the high background organic matter content can

Table 3 Statistical summary for human blood results

Population	Mean/μg L^{-1}	Standard deviation/μg L^{-1}	Median/μg L^{-1}	Range/μg L^{-1}
South African school children, $n = 49$	0.85	0.90	0.39	<0.03–3.82
Middle Eastern men, $n = 9$	2.51	1.11	2.08	1.40–4.35

affect the antimony recovery as we found in this study. Additionally, the age of the population studied and environmental conditions may play a role. Cullen *et al.*[29] found the serum antimony concentrations in Irish infants 2–56 weeks old to be 0.16 to 0.18 μg L^{-1} but the values for whole blood were not reported.

Our Sb concentrations in blood of adults are consistent with the 2.0 μg L^{-1} reported for the UK population[30] and 2.2 μg L^{-1} reported for the Italian population.[31] Gebel *et al.* reported 0.69 μg L^{-1} Sb in blood samples from 48 "unexposed" adults in Lower Saxony, Germany, while the average concentration for the so-called geogenically exposed adults in northern Palatinate (Germany) was found to be 0.89 μg L^{-1}.[32] Their results are not directly comparable to ours since measurements were made by graphite furnace atomic absorption spectrometry (GFAAS) with the reported detection limit of 0.5 μg L^{-1} (very high). Our values are also higher than the 0.64 μg L^{-1} obtained for 67 unexposed office workers in Taiwan,[33] and the 0.258 μg L^{-1} for 31 non-smoking, healthy athletes living in Sweden.[20] Our values are lower than the 2.6 and 10.1 μg L^{-1} reported for seven caster and fourteen formers, respectively, working in the lead battery production industry.[34] Antimony in this study was determined with hydride atomic absorption spectrometry. In a screening of 27 elements in blood samples from 100 healthy volunteers in France, Goullé *et al.*[23] reported median Sb concentration of 0.08 μg L^{-1}. This particular study serves to exemplify the tremendous potential and pitfalls of the ICP-MS in biomonitoring. While a lot of elements can be determined simultaneously, the result for each element needs to be individually validated.

Conclusion

An accurate and precise method is described for the determination of antimony in human whole blood using nitric acid-hydrogen peroxide digestion and a quadrupole ICP-MS spectrometer. The method has a detection limit of 0.03 μg L^{-1} antimony (3σ, $n = 12$). The % recovery ranges from 92% to 104% at various levels of antimony in Seronorm™ trace elements whole blood reference materials. The % RSD was less than 4% ($n = 5$) at all levels. The method was applied to the determination of antimony in the blood of South African school children and adults living in Dearborn, Michigan, USA. The method described here should encourage additional research to evaluate further the use of blood antimony as a biomarker of antimony exposure and in risk assessment.

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