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Analytical

Simplified sample preparation in the simultaneous measurement of whole blood antimony, bismuth, manganese, and zinc by inductively coupled plasma mass spectrometry

Carrie J. Haglock-Adler [a,*], Frederick G. Strathmann [a,b]

[a] ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT 84108, USA
[b] University of Utah School of Medicine, Salt Lake City, UT 84108, USA

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ABSTRACT

Objectives: We developed and validated a simplified sample preparation for the analysis of antimony (Sb), bismuth (Bi), manganese (Mn), and zinc (Zn) in whole blood. This simplification included a reduction in sample volume, removal of a lengthy acidic digestion, and optimization of the internal standard.

Design and methods: Measurement of Sb, Bi, Mn and Zn in whole blood was conducted using inductively coupled-plasma mass spectrometry. Method performance characteristics, including intra- and inter-assay imprecision, accuracy, linearity, AMR, sensitivity, carryover, sample stability and assay stability were determined in accordance with clinical laboratory standards. In addition, analytical and clinical recoveries were assessed to investigate comparability between goat blood matrix and pooled patient blood.

Results: Established assay performance characteristics included inter- and intra-assay imprecision <4.5% and carryover of <0.04% for all four elements, analytical measurement range of 1 to 25 μg/L (Sb and Bi), 1 to 80 μg/L (Mn), and 50 to 1500 μg/dL (Zn), limit of quantification of 1 μg/L (Sb, Bi, Mn) and 50 μg/dL (Zn) (coefficient of variation <14%), proportional bias of 0.96 and constant bias of −0.28 (Sb), 0.94 and −0.45 (Bi), 1.07 and −0.37 (Mn) and 0.96 and +18.05 (Zn) based upon repeat patient samples, proficiency testing samples, and comparison to an outside reference laboratory.

Conclusion: This method overcomes the laborious acidic heat digestion previously used and replaces it with a simplified sample preparation involving an alkaline dilution. The method requires minimal sample preparation with the dilution of alkaline diluent and is validated to quantify Sb and Bi from 1 to 25 μg/L, Mn from 1 to 80 μg/L, and Zn from 50 to 1500 μg/dL in whole blood.

Introduction

Elemental testing in a clinical setting can be broken into two categories — testing for nutritional adequacy of essential trace elements and investigation of potential toxic element exposure. The four elements analyzed in this whole blood panel, antimony (Sb), bismuth (Bi), manganese (Mn) and zinc (Zn), fall under the latter category of exposure. People are most often exposed to these elements either through medications such as parasiticides [1], astringents [2], antacids (Pepto-Bismol) [2], and common cold remedies (Zicam) [4], as well as nutritional supplements containing Mn and Zn [3,4] or occupational exposure in the fields of pigments [1,2], flame-proofing fabrics [1], alloys [1–4], batteries [3], ceramics [3], galvanizing [4], and soldering fluxes [4]. Due to the low concentrations associated with elemental exposure assessments, analytical sensitivity is a concern.

To achieve required sensitivity from whole blood samples with inductively coupled plasma-mass spectrometry (ICP-MS), the previously used extraction method required a laborious chemical digestion involving a time consuming (4+ h) heated acidic digestion of the sample. This aggressive extraction method was required in large part due to limitations in the sensitivity of available instrumentation. Because current instrumentation is considerably more sensitive than previous instruments we developed a simplified and cost-effective method without sacrificing needed sensitivity.

The objectives of this study were as follows: 1) reduce the volume of patient sample required; 2) remove the lengthy acid digestion; 3) reduce the internal standard concentration to achieve better control of the assay and 4) incorporate the measurement of zinc.

Abbreviations: Sb, antimony; Bi, bismuth; Mn, manganese; Zn, zinc; Y, yttrium; In, indium; ICP-MS, inductively coupled plasma mass spectrometry.

* Corresponding author at: ARUP Laboratories, 500 Chipeta Way, Mail Code 209-D02, Salt Lake City, UT 84108-1221, USA. Fax: +1 801 584 5207.
E-mail address: carrie.adler@aruplab.com (C.J. Haglock-Adler).

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Materials and methods

Use of clinical samples and human subjects

Previously analyzed whole blood samples were de-identified and saved for analysis in compliance with the University of Utah Institutional Review Board (IRB #00007275).

Chemicals, reagents, and solutions

Triton X-100 was purchased from VWR (Radnor, PA), EDTA dipotassium salt from Mass Supply (Highland Ranch, CO), and ammonium hydroxide from JT Baker (Center Valley, PA). Nanopure water was from a Barnstead Nanopure Diamond System, from Thermo Scientific (Waltham, MA). Sb, Bi, Mn, Zn, and indium (In) stock solutions (1000 mg/L) were purchased from Inorganic Ventures (Christiansburg, VA). Goat blood, K_2EDTA, was from Lampire Biological Lab (Pipersville, PA).

Sb, Bi, Mn, and Zn stocks were prepared in nanopure water. Calibration standards were prepared in goat blood at 1, 5, 15, and 25 μg/L for Sb and Bi; 1, 10, 40, and 80 μg/L for Mn; and 50, 500, 100, and 1500 μg/dL for Zn. Quality control samples were made in-house from separate elemental stocks spiked into goat blood.

Acidic digestion method

The historic, previously validated sample preparation included diluting 0.5 mL of whole blood with 0.5 mL of concentrated nitric acid in a 10 mL metal-free polypropylene tube. Tubes were closed with vented caps, and placed in the 80 °C heat block to digest for 4 h. Due to workflow efficiency needs and turnaround time expectations, a 2× dilution of each patient sample was also prepared simultaneously in the event that an elevated result required dilution. After the samples were cooled to room temperature, 0.5 mL of 0.10 μg/mL yttrium (Y) in 3.4 M nitric acid (internal standard) and 3.5 mL of nanopure water were added. The mixture was capped, inverted, and vortexed before being introduced to the Perkin Elmer 9000 ICP-MS via a CETAC ASX-500 series autosampler (Omaha, NE), equipped with a 0.5 mm ID probe. The instrument was controlled by Elan software (ver. 3.4). The integrated peristaltic pump introduced the samples via a PFA PTFE Scott spray chamber with a MicroFlow PolyPro-ST nebulizer, followed by a set of platinum interface cones, and standard quartz torch with a 2.0 mm injector. Sb (*m/z* 121), Bi (209), and Mn (55) were monitored, as well as Y at mass 89 as the internal standard. The method consisted of three sweeps, two readings, and three replicates with a dwell time of 200 ms per element. Calibration was achieved with four calibrators for Mn (1–25 μg/L) and three calibrators of Sb (2–20 μg/L) and Bi (1–10 μg/L). A linear through zero curve fit was applied.

Alkaline dilution method sample preparation

Whole blood samples were mixed thoroughly before removing aliquots for analysis. Samples were prepared by adding 4.9 mL of alkaline diluent (1.75% EDTA, 0.1% Triton X-100, 1% NH_4OH, and 1.5 ng/mL indium) to 0.1 mL of patient whole blood, quality controls or calibration standards in a 10 mL metal-free polypropylene tube. The mixture was capped, inverted and vortexed before being introduced to the ICP-MS via a CETAC ASX-500 series autosampler with Glass Expansion Niagara Plus (Pocasset, MA) discrete sampling unit with a 0.25 mL sample loop (1 mm ID). The Niagara Plus uses separate Niagara II software (ver. 2.4.0.0) for control of the unit.

ICP-MS method

The alkaline dilution samples were analyzed using a method developed on an Agilent 7700x ICP-MS configured with collision cell technology. The instrument was controlled by MassHunter software (ver. A.01.02). The sample introduction system was a double walled, Peltier cooled (2 °C) spray chamber with a Micro Mist nebulizer. The samples were introduced with an integrated peristaltic pump and a Niagara Plus sample introduction system (Glass Expansion) equipped with a 0.5 mm ID probe, followed by a set of nickel interface cones under hot plasma (1550 W) condition with a standard one piece quartz torch with a 2.5 mm injector tube. The instrument was operated in a gas mode, with He flowing at 4 mL/min. Sb (*m/z* 121), Bi (209), Mn (55), and Zn (66) were monitored, as well as In at mass 115 as the internal standard added at a concentration of 1.5 μg/L. The instrument was operated in a spectrum mode with a peak pattern of one, three replicates, and 100 sweeps. Each analyte had an integration time of 1 s, while the In internal standard was set at 0.2 second integration time. Calibration was achieved with four calibrators spanning the analytical measurement range (AMR), a reagent blank of diluent only, and a matrix only sample to account for any endogenous elements in the goat blood matrix. A linear curve fit was applied, with an ignored origin and no weighting.

Method validation

The method validation included an assessment of intra- and inter-assay imprecision, accuracy, linearity, AMR, sensitivity, carryover, sample stability and assay stability.

Intra-assay imprecision and inter-assay imprecision were determined using goat blood fortified with Sb, Bi, Mn and Zn. Intra-assay imprecision was calculated based on the analysis of one sample, prepared and injected 20 times within a single batch. For inter-assay imprecision, samples were analyzed in quadruplicate for 5 days. Accuracy was determined by the analysis of whole blood samples comprised of previous proficiency test samples, patient samples, and fortified samples. Sb, Bi, and Mn patient samples were compared to the in-house digestion method, while Zn samples were compared to an outside reference laboratory. A total of 40 samples for each element were analyzed.

Linearity and AMR were assessed by analyzing fortified goat blood samples over a range of 0–50 μg/L Sb and Bi, 0–80 μg/L Mn, and 31–1690 μg/dL Zn. Prepared samples were run in quadruplicate in a single batch. To establish the limit of quantification (LOQ) goat blood was either fortified with elemental stock or diluted with 0.1% BSA to the desired concentration (1 μg/L Sb, Bi and Mn and 50 μg/dL Zn) and analyzed in quadruplicate over 5 days. To determine the limit of blank (LOB) a diluent only sample was also analyzed in quadruplicate over 5 days with the LOB equal to the mean plus 1.65 times the standard deviation. Calculation of the LOB in this manner provides a minimum concentration for the matrix additive (e.g., goat blood) to ensure that the reagent blank and the matrix only sample used in the calibration do not overlap in CPS which can lead to negative patient values. A dilution strategy with nanopure water was validated at 2×, 5×, 10×, and 20×.

Carryover was assessed at 100 μg/L for Sb, Bi, and Mn and at 2000 μg/dL for Zn. Three sets of two low concentration samples (5 μg/L Sb, Bi and Mn and 50 μg/dL Zn) preceded by two high concentration samples were analyzed. The percent carryover was then calculated using the following equation: Percent carryover $= 100 * [(L1_{AVG} - L2_{AVG}) / H2_{AVG}]$.

To ensure that elemental concentration was stable in whole blood matrix, samples were fortified to create low and high concentration samples. Samples were stored at room temperature (25 °C), refrigerated (2–8 °C) and frozen (−20 °C) for 14 days. Quantification was conducted over the course of two weeks. Three freeze/thaw cycles were also performed on samples stored frozen; analysis was performed with each thaw. In addition, the stability of the assay and instrument was tested using a pool of fortified goat blood independently prepared and analyzed up to a maximum batch size of 168.