Hydra AF











Determinación de Mercurio en sangre mediante Fluorescencia atómica vapor frío AFAS laboratorio



Introduction

Mercury is a toxic element that can be inhaled as vapor or pass through the skin and into the blood stream. Compounds of mercury can enter the body through the lungs, skin or by ingestion. Repeated exposure to mercury has adverse health effects whose symptoms are well documented. Individuals at high risk of exposure or who are suspected of mercury intoxication are typically monitored through analysis of blood and urine samples. The mercury blood test will detect all types of mercury but because mercury remains in the bloodstream for only a few days the test should be performed soon after exposure. The urine mercury test only measures inorganic and elemental mercury as organic forms are not excreted.

This application note describes sample preparation and analysis of blood samples using the Hydra AF mercury analyzer. The Hydra AF employs cold vapor atomic fluorescence and is ideally suited for the determination of mercury ranging from <10ug/L up to the elevated levels sometimes found as a result of occupational exposure.

Experimental

Sample Preparation

0.1 mL of blood was pipetted into a 12mL polypropylene test tube. Next, 3.2mL of concentrated sulfuric acid was added and the mixture allowed to sit at room temperature for 20 minutes to digest the sample. De-ionized water¹ and 5% potassium permanganate were added to each sample tube and tubes were held



at 50°C for 30 minutes to oxidize mercury to mercuric (+2) valence. Samples were cooled to room temperature and sodium chloride:hydroxylamine sulfate (12%:12%) solution was added to remove excess permanganate.

¹ The de-ionized water mentioned here was added to samples only and excluded from standard cups. The water added is to compensate for the standard solution added to calibration standard cups. The standard cups contained 4mL of mercury standard solution in 2% hydrochloric acid and no blood. The water added contained 50ppm anti-foaming agent (Leeman Labs #606-00008



Table 1: Reagent Additions

Reagent	Samples	Standards
Mercury standard	0.0mL	4.0mL
Water with antifoam	4.0	0.0
H2SO4	3.2	3.2
5% KMnO4	0.4	0.4
NaCl:NH2OH·SO4	0.5	0.5

Sample Analysis

The Hydra AF mercury analyzer is a continuous flow system where sample and reductant, in this case 10% SnCl₂, are pumped into a gas/liquid separator. There the mercury in the sample is reduced to elemental mercury. It then enters the gas phase and is carried to the spectrometer. Instrument parameters are provided in Table 2.

Table 2: Instrument Parameters

Parameter	Value	
Gas flow	0.3 LPM	
Pump speed	10 mL/min	
Rinse time	60 sec.	
Uptake time	20 sec.	
Integration time	30 sec.	

Samples were mixed and then diluted 1:6 with 2% hydrochloric acid prior to analysis. The calibration standard concentrations were entered as the original values before reagent addition and dilution (0.0, 0.2, 1.0, 2.0 & 6.0 μ g/mL). Sample concentrations were reported with 40-fold volume corrections applied [0.1ml \rightarrow 4.0 mL]. Figure 1 shows the calibration curve. The fit type is linear with a correlation coefficient of 0.999834. Two replicates run on each standard show excellent precision throughout.

WinHg Database 1.5 File Utility Help ■ RN→ RN→ Dataset/Proto BLOOD4 /BLOOD4 Protocol BLOOD4 Protocol Line info Cal Curve Report Ctrl Chart Viewer Reset Intensity A 33969 Calib Coeffs B 1.77270e-4 Accepted C -4.71810e-2 Calibrated New Cal .999834 ✓ Accepted New Update Coeffs Accept Spike Coeffs Include S1 Rep 1 ▼ 2 ▼ 3 □ 4 □ 5 □ 16-Feb-06 10:43 Conc. 6.00 Dev. Mean SD or %RSD Rep 1 Rep 3 Conc. Calc. Rep 2 01 .00000 -.023 -.023 137 147 127 -.022 1.56% 1287 1259 .20000 .178 1273 03 1.0000 .991 -.009 5854 0.53% 5877 5832 04 2 0000 2.08 .079 11997 0.24% 12016 11976 05 6.0000 33969 0.62% 34118 33820 NA <

Figure 1: Linear Calibration

Reference Materials

Three control samples of lyophilized whole blood were obtained from Bio-Rad Laboratories, Irvine, CA. Results for samples are provided in Table 3.

Table 3: Control Samples

Lyphochek	Lot #	Result	Reference Mean	Reference Range ²
Level 1	36681	10.7 _µ g/L	9.6 _µ g/L	7.7-11.6 _µ g/L
Level 2	36682	37.8	39	31-47
Level 3	36683	75	73	58-87

Conclusions

The Hydra AF mercury analyzer provides accurate measurement of Hg in whole blood without the need for microwave digestion of samples.

¹ Reference data were established for Bio-Rad by inductively coupled plasma mass spectrometry (ICP-MS).