BUCK Scientific

Application Note #AA3004

Analysis of Whole Blood for Trace Lead (Pb) by Graphite Furnace AAS

Toxic effects from heavy metals (Pb, As, Se, Cd, Bi, Sn, Tl, and Hg) have been characterized in medical literature for decades. Originally, complex extractions were performed to isolate the poisonous components from the complex biological matrices: blood, serum, urine and tissues. Recently, optical emission and atomic absorption spectroscopy techniques were developed to determine these toxic elements. As analytical instrumentation performance improved, real limits of detection were also improved. The advent of Inductively-Coupled Plasma (ICP) and Graphite Furnace AAS (GFAAS) brought the limits of analysis into the parts-per-billion (PPB) range for many matrices. This capability has been invaluable in screening blood and urine samples from pediatric and chronically ill patients to track the exposure and elimination of lead.

The determination of trace lead in blood samples is very important, particularly in "third world" and urban environments, where environmental pollution, lead-based paints, gasolines, and poor plumbing systems increase the exposure of lead in various forms. The standard clinical procedure for the measurement of lead in blood is very simple and is based on the use of highly sensitive GFAAS instrumentation. The procedures listed below and the data were developed on and obtained from the Buck 210VGP Atomic Absorption System with the Model 220 Graphite Furnace option.

Sample / Standard Preparation:
There are several procedures for the quantitative and reproducible preparation of Whole Blood samples prior to atomic analysis. Below is the most popular technique:

(1) Collect Blood and preserve with Heparin, EDTA, or Oxalate.
(2) Dilute 100µL of sample with 400µL of "Diluent" (contains 0.25% Triton X-100, 2000 ppm ammonium dihydrogen phosphate, and 750 ppm magnesium nitrate). These "matrix modifiers" serve to aid hemolysis, sequester the Pb, and increase atomization efficiency.
(3) Make 0, 0.01, 0.05, 0.1, 0.25, and 0.5 mg/L lead standards in diluent.
(4) Analyze 20µL loads with the following GFAAS conditions: 283.3nm wavelength, 7A slit, D2 background correction, Peak Height, Argon purge (~50ml/min), Auto-Zero off, grooved furnace tube. Dry: Ambient to 125°C in 15 second ramp, 5 second hold. Ash: 125°C to 600°C in 45 second ramp, 20 second hold. Atomize: 600°C to 2400°C in fast ramp or step, 5 second hold.

This technique provides a well-matched calibration equivalent to 40, 200, 400, 1000, and 2000µg/L (ppb) lead in the original blood sample. The precision ranges from ~3% relative at the upper limit to ~8% at the low end, with a method detection limit of 25µg/L in blood calculated from the blank variance taken at 2-sigma.

SIC: 801, 8062, 8071

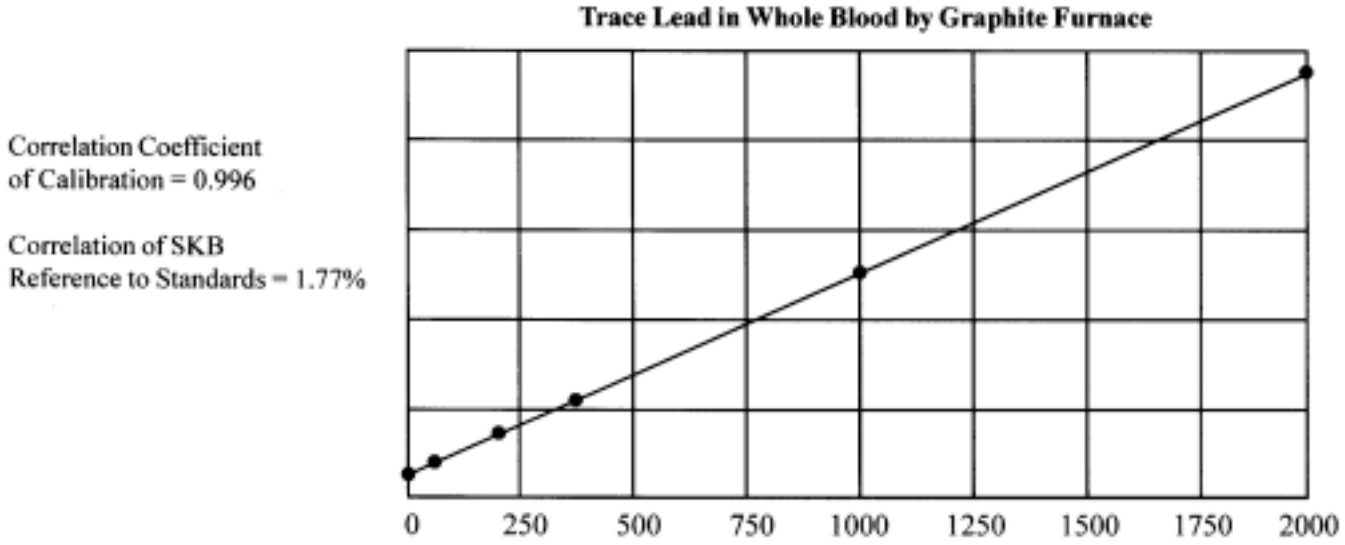
Analysis of Whole Blood for Trace Lead (Pb)

Samples: "blank" pooled Blood sample (Red Cross), no Lead.
"real" pediatric Blood collection (Newark Children's Hospital)
"spiked" reference Blood (S-K Bioscience, asssay = 50.6 PPB)

Results: Values are µg/L (PPB) in the original diluted samples:

Sample ID	Trip. Readings	Average	R.S.D.
"blank"	23, 27, 26	25.3	6.7%
PED-113	169, 181, 166	172	3.8%
Ref-50	53, 47, 49	49.7	5.0%

Standard Calibration Curve: (linear regression)



The superb precision and accuracy shown by the above data are typical of the quality optical components and engineering design of the Buck AA systems. The industrially rugged nature of this design also allows the instrument to be used in a wide range of environments, from Hospital R&D Centers, to Clinical Testing Laboratories with much ease and confidence.

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