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Application News

No. AD-0121Ph

Quantitative Bioanalysis / AA-7000G

Direct Determination of Pb in Whole Blood by Graphite Furnace Atomic Absorption Spectrophotometry (GF-AAS)

Chua, Modesto[1], Parreñas, Thelma[1], Kierulf, Arkaye[1], Ignacio, Austin[1], Argamino, Cris Ryan[2], Castaños, Sara Jane[2] and Torres, Arturo[2]

❑ Introduction

Lead (Pb) is a widely used, cumulative heavy metal which affects numerous body systems, including neurological, hematological, gastrointestinal, cardiovascular and renal [1]. At present, ten (10) µg/dL (micrograms /deciliter) or 100 ppb (parts per billion) was adopted by the Centre for Disease Control & Prevention (CDC) in 1991 as an advisory level for environmental and educational intervention [2]. Detection of Pb in blood can be performed using several methods including atomic absorption spectrophotometry (AAS), anodic stripping voltammetry (ASV) and inductively coupled plasma mass spectrometry (ICP-MS). The method of choice will rely on various aspects including analytical capability (i.e. LOD, LOQ), costs, and technical requirements (i.e. sample preparation) [3]. This application news will report on the analysis of Pb in whole blood using the Shimadzu AA-7000G (Graphite Furnace Atomizer) and the Platform-type Graphite Tube. The method presented is a quick way to determine Pb in whole blood as the sample was mixed with a matrix modifier solution prior to GF-AAS analysis.

❑ Experimental

Preparation of Matrix Modifier, Standards and Samples

In a 100 mL volumetric flask, 5 mL of 10% Triton X-100, 2 mL of NH_4PO_4 and 4 drops of 70% HNO_3 acid were mixed and diluted to volume with deionized water to form the matrix modifier. To prepare a multi-point calibration curve, 0, 50, 100, 300, and 600 ppb Pb working standard solutions were prepared in 1% HNO_3. The final standard solutions were prepared by mixing 100 µL each of the working standard solution and 900 µL of matrix modifier in the autosampler vessels to produce 0, 5, 10, 30, and 60 ppb. These standard solutions were set aside until the bubbles were dissipated.

The samples were prepared by mixing 100 µL of whole blood (with anti-coagulant) with 900 µL matrix modifier. On the other hand, the spiked recovery samples were prepared by mixing 100 µL blood sample, 100 µL working standard solution and 800 µL matrix modifier.

Table 1: Instrument and analytical conditions

AAS	AA-7000 with GFA-7000
Lamp current	10 mA
Wavelength	283.3 nm
Slit width	0.7
Measurement mode	BGC-D2
Graphite tube	Platform type
Autosampler	ASC-7000
Injection volume	10 µL
Workstation	WizAArd version 5.02

Table 2: Temperature Program

Pb

	Temp.	Time (sec)	Heat Mode	Sensitivity	Gas Type	Flow Rate
1	60	3	RAMP	☐	#1	0.10
2	120	20	RAMP	☐	#1	0.10
3	250	10	RAMP	☐	#1	0.10
4	700	10	RAMP	☐	#1	1.00
5	700	10	STEP	☐	#1	1.00
6	700	3	STEP	☑	#1	0.00
7	2000	3	STEP	☑	#1	0.00
8	2500	2	STEP	☐	#1	1.00

Atomization Stage: Step 7

❑ Results and Discussion

Figure 1 shows the Pb calibration curve whereas Figure 2 shows the peak profiles of the standard solutions.

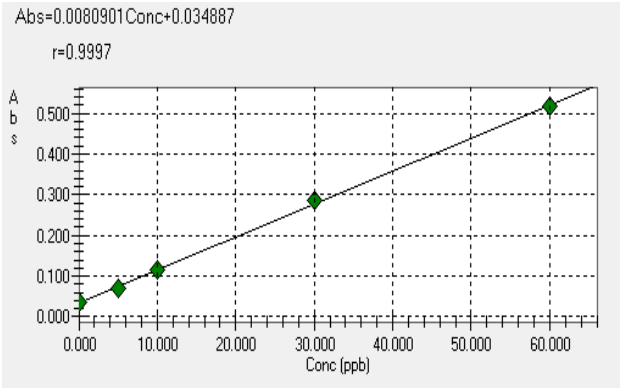

Figure 1: Pb calibration curve

[1]Philippine Institute of Pure & Applied Chemistry, [2]Shimadzu Philippines Corporation

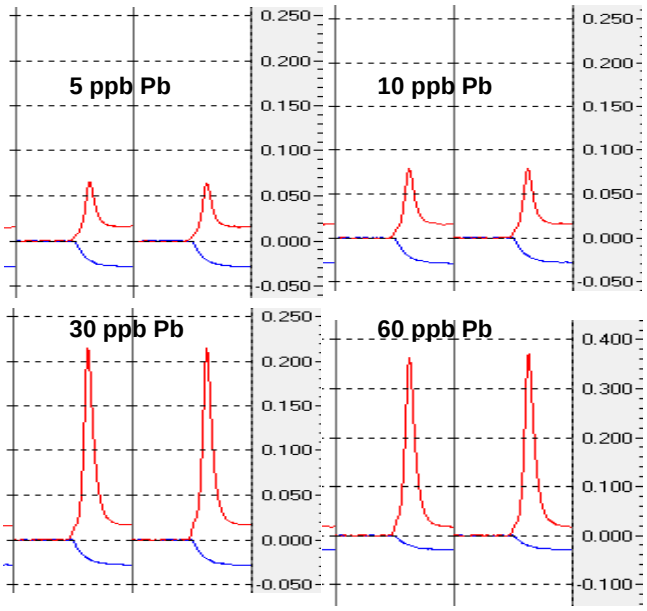

Figure 2: Calibration Standard Peak Profiles

Good percentage recoveries (% R) were obtained for the spiked samples as shown in Table 3.

Table 3: Spiked percentage recovery

Sample + Pb Spike	% R	% RSD
Whole Blood +10 ppb Pb	100.06	5.63
Whole Blood + 30 ppb Pb	109.10	5.71
Whole Blood + 50 ppb Pb	99.65	3.59

The method uses platform-type tube, which allows Pb to be atomized only when the inside of the tube has reached atomization temperature. In addition, the ammonium phosphate-Triton X-100 matrix modifier stabilizes Pb atoms during pyrolysis (>600ºC) and also breaks-up and increase the volatility of the interfering sample matrix.

The platform-type tube, also known as an L'vov platform, is used with integrated peak areas for absorbance measurements [3] through the WizAArd workstation. Figure 3 compares the peak profiles of the sample (whole blood) as well as the same sample spiked with 30 ppb Pb.

The use of the combined approaches above decreases the effect of interference from the sample matrix, which can be verified from the good spiked percentage recovery values as shown in Table 3.

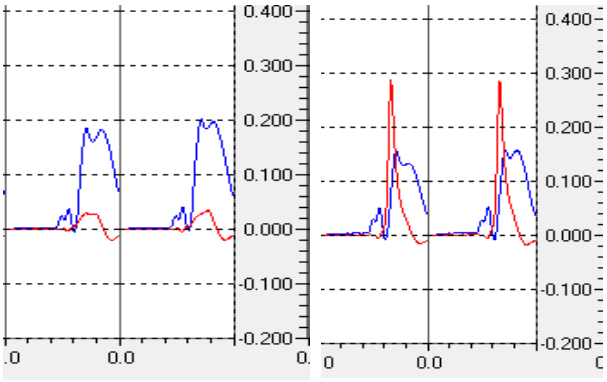

Figure 3: Peak profiles of whole blood (left) and whole blood spiked with 30 ppb Pb (right)

❑ Conclusions

Direct determination of Pb in whole blood using the platform-type graphite tube together with a matrix modifier can be performed accurately on Shimadzu AA-7000 with GFA-7000. This combination provides for an effective way to analyze a volatile element in a complex biological sample matrix. The graphite tube design allows sample atomization to take place under a more uniform temperature condition, whereas the matrix modifier releases and prevents loss of the target element while minimizing matrix interference. The standard solutions used, which were below the CDC Blood Lead Level limit of 10 μg/dL (100 ppb), showed good linearity for the calibration curve (r=0.9997). The good spiked recovery results (99-109%) also provided additional evidence of the instrument and method's suitability for the effective analysis of Pb in whole blood sample.

❑ References

[1] Brief guide to analytical methods for measuring lead in blood. World Health Organization. 2011. (http://www.who.int/ipcs/assessment/public_health/lead_blood.pdf).
[2] Lead Toxicity: What Are the U.S. Standards for Lead Levels? (http://www.atsdr.cdc.gov/csem).
[3] The Lead Laboratory (http://www.cdc.gov/nceh/lead/publications/ 1997/pdf/c1.pdf).

Disclaimer: The Shimadzu AA-7000G system and the data in this Application News are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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SHIMADZU Philippines Corporation
11F Sun Life Centre, 5th Avenue corner Rizal Drive, Bonifacio Global City, Taguig City 1634 Philippines
www.shimadzu.com.ph
Tel: +632 869-9563 Fax No: +632 519-9285