APPLICATION NOTE

Atomic Absorption

Author:

Cynthia Bosnak

PerkinElmer, Inc.
Shelton, CT 06484 USA

Using THGA and Zeeman Background Correction for Blood-Lead Determination in Customer-Validated Applications

Introduction

A reliable clinical-use instrument that facilitates the accurate determination of blood-lead levels in customer-validated applications is in the interest of occupational safety and public health. The CDC (Centers for Disease Control) currently requires action for any blood-lead level concentrations above the current 10 µg/dL guideline.[1] However, there is no safe threshold for lead in blood and it is important that any customer-validated application incorporate a clinical instrument that provides accurate and precise measurements.

Validated applications determining whole blood levels are generally performed using graphite furnace atomic absorption spectroscopy (GFAAS). GFAAS is cost effective, allows for detection limits well under the blood-lead level action guideline, and requires less operator training than more advanced elemental techniques.[2] In this study, we will demonstrate the applicability of the PerkinElmer® PinAAcle™ 900T atomic absorption spectrometer (Figure 1) using the stabilized temperature platform furnace (STPF) and transversely-heated graphite atomizer (THGA), for use in customer-validated applications to determine lead amounts in blood samples. Aqueous calibration standards were used instead of matrix-matched blood standards or the method of additions for calibration. This simplifies and minimizes analysis time.

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Experimental

Preparation of Reagents

All reagents, standards and samples were prepared with ASTM® Type I deionized water (18 MΩ • cm). Concentrated nitric acid (69-70%), HNO_3, was trace metal grade (TMG) or better.[3]

- **10% Triton® X-100 Stock Solution:** Weigh 10 grams of Triton® X-100 wetting agent (Part No. N9300260) directly into a 125 mL LDPE bottle. Add deionized water up to 100 grams. Shake well to mix thoroughly.
- **Autosampler Rinse Bottle Solution:** Almost fill the 2 L autosampler rinse bottle with deionized water. Add 4 mL of concentrated nitric acid, and 100 µL of 10% Triton® X-100 solution. Shake well.
- **Diluent/Matrix Modifier Solution:** Into a 60 mL LDPE bottle, pipette 1 mL of 10% Ammonium Dihydrogen Phosphate, $NH_4H_2PO_4$, matrix modifier (Part No. N9303445) and 2.5 mL of the 10% Triton® X-100 Stock Solution, and 0.1 mL of concentrated nitric acid. Dilute to 50 mL with deionized water.
- **1% HNO_3 solution:** Add approximately 500 mL of deionized water to a 1 L plastic volumetric flask. Pipette 10 mL of concentrated nitric acid and dilute to volume with deionized water.
- **Intermediate Standard Solution (10 mg/L):** Pipette 1 mL of 1000 mg/L Lead stock standard (PerkinElmer Pure, Part No. N9300175) into a 125 mL LDPE bottle and bring to 100 g with 1% HNO_3. Prepare monthly.

Standard and Sample Preparation

Dilute the 10 mg/L intermediate stock standard to 100, 200, 400, and 600 µg/L secondary standards by adding 1, 2, 4, and 6 mL, respectively, of the 10 mg/L intermediate stock and bringing up to volume in clean 100 mL plastic flasks with 1% HNO_3.

To make the calibration standards: Into clean 1.2 mL autosampler cups (Part No. B0510397), pipette 100 µL of each secondary solution into 900 µL of diluent/modifier solution. Flush the pipetter up and down 5-10 times to completely mix the solution in the autosampler cup. Repeat the procedure using the 1% HNO_3 solution for the blank.

NIST® Trace Elements in Caprine Blood (SRM 955c) and LyphoChek Whole Blood Metals Controls Levels 1, 2, and 3 (BioRad, Hercules, CA) were used as samples. Samples were diluted directly into the 1.2 mL autosampler cups by pipetting 100 µL of each blood sample into 900 µL of the diluent/modifier solution and mixing as above.

Instrumentation

A PinAAcle 900T flame and longitudinal Zeeman furnace atomic absorption spectrometer was used for all measurements. A PerkinElmer Lumina™ single-element Pb hollow cathode lamp (Part No. N3050157) was used as the light source and argon was the normal gas type. The PinAAcle 900T instrument settings are listed in Table 1 and the furnace program used for all samples is listed in Table 2.

Figure 1. PinAAcle 900T atomic absorption spectrometer with AS 900 furnace autosampler.

Table 1. **Instrument Settings for the PinAAcle 900T.**

Parameter	Value
Wavelength:	283.3 nm
Slit:	0.7 nm
Lamp Current:	10 mA
Integration Time:	4 s
Calibration Type:	Linear through zero
Replicates:	2
Standard Units:	µg/dL
Sample Units:	µg/dL
Sample Volume:	12 µL
BOC:	2 s

Table 2. **Furnace program for measuring Pb in blood samples using a PinAAcle 900T with THGA tubes.**

Step	Temp. (˚C)	Ramp (sec)	Hold (sec)	Internal Flow	Read Step	Gas Type
1	120	5	10	250		Normal
2	140	5	10	250		Normal
3	200	10	10	250		Normal
4	700	10	20	250		Normal
5	1500	0	4	0	X	Normal
6	2450	1	3	250		Normal

**Injection Temperature: 110 ˚C*

Standard pyrolytically-coated THGA tubes (Part No. B0504033) with integrated platforms were used for all analyses. The unique patented design of the THGA tubes provides consistent heating and high atomization efficiency for all elements including refractory elements. The tube and integrated platform are machined from a single block of PerkinElmer exclusive, high-density graphite. The transverse heating of the tube ensures a uniform temperature distribution along the length of the tube, thereby significantly reducing condensation of the matrix components and memory effects. The longitudinal Zeeman-effect background correction provides accurate correction without the loss of light associated with transverse Zeeman systems.

The TubeView™ furnace camera on the PinAAcle 900T was used to adjust the pipette tip to the most appropriate depth (Figure 2a) and to watch for matrix buildup on the platform should it occur. The camera was also used during method development to verify the drying steps and ensure that no sample boiling or splattering occurred (Figure 2b).

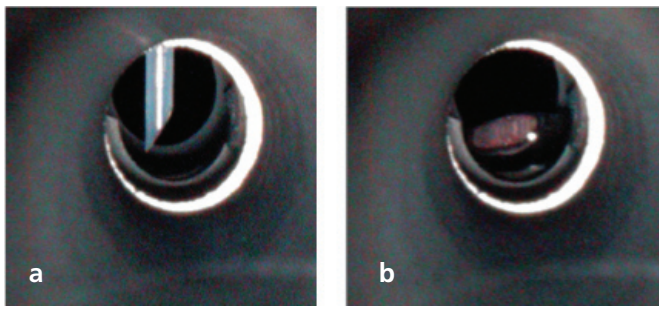

Figure 2. a) AS 900 autosampler alignment as seen using the TubeView furnace camera; b) blank sample inside the THGA during method development.

Results and Discussion

Aqueous calibration standards are more convenient to use than multiple standard additions steps for Pb in blood analyses. It provides for less operator error, lower cost, and shorter analysis times than with standard additions or matrix matched standards.[4] An overlay of the peak plots for an aqueous Pb standard (red) and a blood reference material (blue) taken on the PinAAcle 900T are shown in Figure 3. As expected, in the blood matrix, Pb volatilization is delayed relative to the Pb in the standard. However, with the STPF conditions used, all Pb atoms are volatilized into a steady-state temperature environment, regardless of appearance time. As a result, each Pb atom contributes equally to the atomic absorption process permitting the use of simple aqueous standard calibration with resulting accuracy and precision.

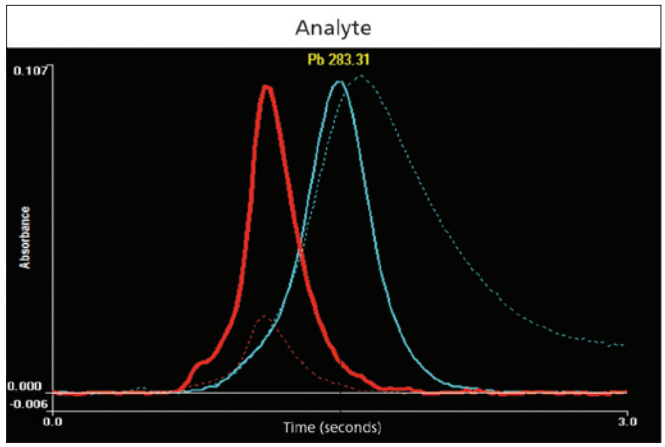

Figure 3. Overlay of an aqueous standard (red lines) and a blood reference material (blue lines). Solid lines are the analytical signal after background correction (AA-BG); dotted lines are the background signal (BG).

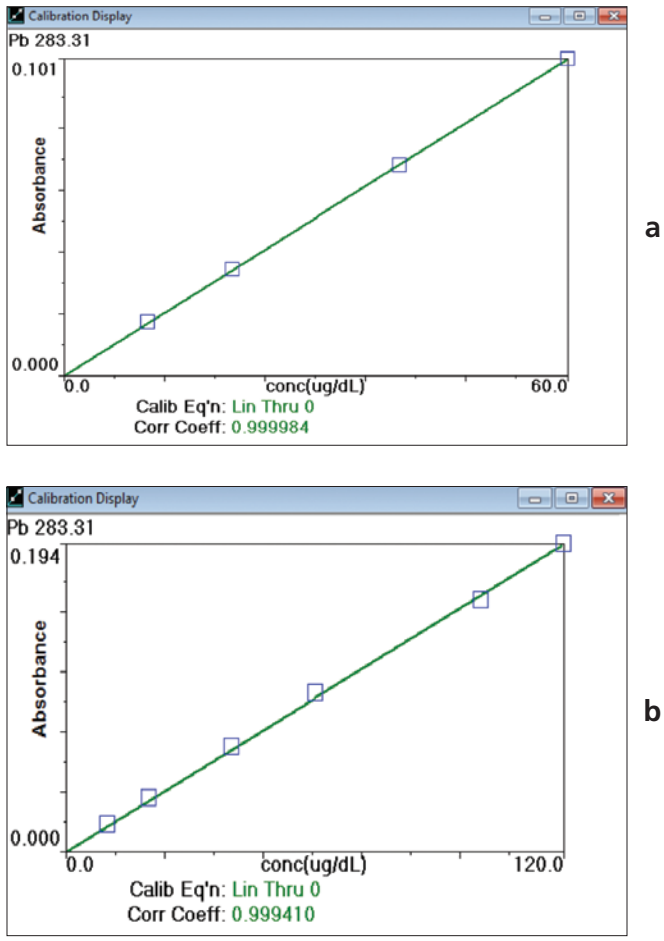

Figure 4. a) Linear calibration curve for Pb samples up to 60 µg/dL. b) Linear calibration curve for Pb samples up to 120 µg/dL.

Although for this analysis, the instrument was calibrated up to 60 µg/dL (Figure 4a), it can be shown that the instrument is linear up to 120 µg/dL or higher (Figure 4b). This provides a means of minimizing the number of sample dilutions that are required for high-level samples. Multiple measurements of the blood diluent solution produced an estimated detection limit of 0.23 µg/dL (3SD).

The NIST® standard reference material 955c, Toxic Metals in Caprine Blood, showed recoveries ranging from 98-102% for all four levels (Table 3). The tri-level BioRad LyphoChek Control samples showed recoveries from 100-106% (Table 4). All experimental values easily fell into the reference value range.

Table 3. **Results for NIST® SRM 955c using aqueous calibration standards and GFAAS.**

Sample	Reference Value (µg/dL)	Experimental Value (µg/dL)	Recovery (%)
Level 1	0.424 ±0.011	0.431	102
Level 2	13.950 ±0.080	13.8	99
Level 3	27.76 ±0.16	27.6	100
Level 4	45.53 ±0.27	44.8	98

Table 4. **Results for tri-level BioRad LyphoChek Control samples.**

Sample	Reference Value* (µg/dL)	Range (µg/dL)	Experimental Value (µg/dL)	Recovery (%)
Level 1	9.58	7.67-11.5	9.83	103
Level 2	27.7	22.2-33.3	29.4	106
Level 3	54.0	43.2-64.8	54.0	100

** BioRad reference values represent an average from laboratories using ICP-MS.*

The AS 900 furnace autosampler was used to ensure accurate delivery of the sample. The AS 900 autosampler probe did not require any cleaning or maintenance between analytical runs to prevent carryover from high-level samples. It can also provide automated dilution of samples outside of the calibration range, if necessary.

Conclusions

An example of how our instrument can be used in a customer's clinically validated application for lead determination was presented here. The speed and accuracy of a customer-validated application is made possible by using the STPF concept, THGA system and detection with the PinAAcle 900T atomic absorption spectrometer. The linear dynamic range allows measurement throughout a large range of sample concentrations from well below the critical threshold of 10 µL/dL to much higher concentrations that may be needed for both clinical and research applications. The PinAAcle 900T atomic absorption spectrometer is suitable for clinical use in customer-validated applications for the measurement of lead in blood.

References

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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