Determination of Lead in Blood by GFAAS–Deuterium and Zeeman Background Correction

Application Note

Atomic Absorption

Authors

Christine Flajnik

Doug Shrader

Introduction

Before the mid-1960s, the perceived toxicity level of lead in blood was 60 µg/dL. However, the blood lead level considered to indicate lead poisoning has fallen steadily since then. Current goals are to reduce children's blood lead levels to below 10 µg/dL [1]. The first techniques used for determining lead in blood were flame atomic absorption methods. The actual sample analysis by flame atomic absorption was extremely fast. The sample preparation, however, was very time consuming. A chelating reagent, ammonium pyrrolidine dithiocarbamate (APDC), was added to a 5.0 mL blood sample. The purpose of the chelation was to make the lead soluble in an organic solvent. Triton X-100, a surfactant, was added to haemolyze the blood. Finally, the sample was extracted with methyl isobutyl ketone (MIBK). The organic extractant was collected and aspirated into the flame. The 217.0 nm wavelength was commonly used [2].

The large sample volume and tedious sample preparation required for the analysis by flame atomic absorption precipitated the advent of 'semi-flame' techniques. In the boat technique, 0.02 mL of blood was accurately measured into a tantalum boat. After drying the sample externally, the boat was inserted into a flame. A transient signal was produced. Though the sample volume was decreased and sample preparation was minimal, reproducibility was a problem. The reproducibility of results depended on where the sample was weighed into the tantalum boat and where the boat was placed into the flame [2].

This method was further developed by Delves, who replaced the tantalum boat with a nickel crucible. The heated sample vapor was directed into a hole in the lower side of a nickel absorption tube situated above the flame. The absorption tube material was later changed to either quartz or ceramic. This technique, called the "Delves Cup" technique, still suffered from reproducibility problems [2]. Both techniques were limited by the lifetime of the sample receptacles and required constant standardization due to degradation of the tantalum boat and nickel crucible.

Agilent Technologies

By 1971 the first "flameless" atomic absorption technique was employed for the determination of lead in blood [3]. The burner head used for flame atomic absorption analysis was replaced with a workhead holding a single electrode (carbon rod) clamped between two water cooled terminals. A power unit supplied current across the workhead assembly and programmed to control temperatures for drying, ashing, and atomizing the sample injected on the carbon rod. Deuterium background correction was later added. The ability to program dry and ash temperatures and times plus deuterium background correction eliminated most of the interferences from the complex blood matrix [4]. The blood samples were prepared by solvent extraction, TritonX-100 dilution or were directly injected. For direct injection techniques as little as 1.0 µL of sample was needed. All samples were manually injected. The carbon rod atomizer was 30–40 times more sensitive than the previous techniques.

The carbon rod atomizer preceded the modern day graphite furnace. Today a graphite tube situated between two electrodes is used. A power supply is still needed to supply current for sequential programming of dry, ash, and atomize steps. The graphite tube allows more sample to be injected into the tube resulting in better reproducibility and sensitivity. Chemical modifier can be added to reduce matrix effects. Atmospheric interferences were eliminated by enclosing the sample in the graphite tube in inert argon gas. More recently the application of Zeeman background correction became possible [5].

As the perceived toxicity level of lead decreased, graphite furnace atomic absorption permitted easy determination of the lower levels. In 1991 the Centers for Disease Control (CDC), Atlanta, GA, came to the conclusion that selecting a single blood lead concentration for the "threshold" limit was unacceptable. Instead, each child was put into a specific class based on the blood lead level found. The corrective action was also based on that amount. Levels ≤ 9.0 µg/dL require no corrective action [1].

Recently, the question has arisen as to whether Zeeman background correction should be used instead of deuterium background correction. Some feel that the deuterium background corrector cannot be used to accurately and precisely correct for the complex blood matrix. However, there are no spectral interferences from blood requiring Zeeman background correction. High speed deuterium instruments have been successfully used for deter-mining lead in blood since the 1970s.

Two basic types of graphite tubes are used in graphite furnace atomic absorption spectroscopy. The first is a partition tube, which is used for wall atomization. It is called a partition tube because on either side of the injection port are rings to contain the sample to the middle of the tube. The tube is heated by the power supplied to it for drying, ashing, and atomizing [5].

The second type is the plateau tube, which is used for platform atomization. The plateau tube is a special tube designed to accept a graphite platform onto which the sample is injected. Also, a modified partition tube will accept "forked" graphite platforms. In both cases the platform is not heated directly but rather by heat transfer from the tube. Therefore, the platform temperature lags behind the tube tempera-ture potentially reducing interferences and back-ground [6].

The following study was based on a method developed by Dr. Patrick J. Parsons at the New York State Department of Health in Albany, NY. The procedure permits blood samples to be run against aqueous calibration standards eliminating the need for standard additions. The aqueous standard and blood sample preparation are taken from this method [7].

This work compares the accuracy and precision of Zeeman background correction and deuterium background correction for the determination of lead in blood. Platform atomization (forked platform tube) and wall atomization (partition tube) are also evaluated.

Experimental

Instrumentation

The instrumentation used for the determination of lead in blood was an Agilent SpectrAA-400Z with Zeeman background correction and an Agilent SpectrAA-400P with deuterium background correction. Both spectrometers were equipped with an Agilent GTA-96/96Z graphite tube atomizer and PSD-96 programmable sample dispenser. Inert gas was high purity argon.

Reagents Required

- Triton X-100
- Ammonium Dihydrogen Phosphate ($NH_4H_2PO_4$), a grade suitable for trace metal analysis.
- Concentrated Nitric Acid (HNO_3), a grade suitable for trace metal analysis.

Reagent Preparation

1. 10% TritonX-100 (v/v) - Pipet 10 mL of TritonX-100 into ~70 mL D.I. water. Stir for ~1 hour. The solution may need to be gently warmed or sonicated for complete dissolution. Make up to 100 mL. (Stable for 1 month.)
2. 20% $NH_4H_2PO_4$ (w/v) - Dissolve 20 g of $NH_4H_2PO_4$ in ~75 mL D.I. water. Make up to 100 mL. (Stable for 6 months.)
3. Working Modifier - To ~400 mL D.I. water add 25 mL 10% TritonX-100 solution, 5.0 mL 20% $NH_4H_2PO_4$ solution and 1.0 mL conc. HNO_3. Make up to 500 mL. (Stable for 3 weeks.)

   Note – If absorbance values start to degrade, make a fresh solution of working modifier as your first corrective action.

Standards Required

1000 µg/mL stock Lead Solution (Pb) – Atomic Absorption standards can be purchased from any major chemical supplier.

1. 10 µg/mL intermediate Pb standard - Pipet 1.0 mL stock Pb standard and 2.0 mL conc. HNO_3 into ~75 mL D.I. water in a 100 mL volumetric flask. Carefully make up to volume (100 mL).
2. Calibration Standards: All prepared weekly in 2% v/v HNO_3. Final volumes are 100 mL, made in volumetric flasks utilizing glass pipettes.

   - 100 µg/L - Pipet 1.0 mL intermediate standard and dilute to 100 mL with 2% v/v HNO_3
   - 200 µg/L - Pipet 2.0 mL intermediate standard and dilute to 100 mL with 2% v/v HNO_3
   - 300 µg/L - Pipet 3.0 mL intermediate standard and dilute to 100 mL with 2% v/v HNO_3
   - 400 µg/L - Pipet 4.0 mL intermediate standard and dilute to 100 mL with 2% v/v HNO_3
   - 600 µg/L - Pipet 6.0 mL intermediate standard and dilute to 100 mL with 2% v/v HNO_3

Blood Samples

Blood controls were purchased to be used as blood samples. Controls were prepared per included instuctions. Two different sources of blood controls were used.

1. Goat Blood - Obtained from New York State Department of Health, Wadsworth Center Lead Poisoning Laboratory, Empire State Plaza, Albany, NY 12201-0509.
2. Human Blood - Obtained from Bio-Rad Laboratories, ECS Division, 3726 E. Miraloma Avenue, Anaheim, CA 92806.

Working Standard and Sample Preparation

All calibration working standards and blood controls were prepared in the same manner. They should be prepared fresh daily. A glass 1.0-mL pipette (graduated in 100 µL increments) was used to add 900 µL of the working modifier to a 2.0-mL graphite furnace sample dispenser cup. Next, 100 µL of the blood control or calibration standard was added to the working modifier. The blood controls and standards were pipetted with a 100-µL micropipettor. All working solutions were carefully mixed with a transfer pipet to insure homogeneity.

Results and Discussion

Table 1 lists the instrument parameters used for both Zeeman background correction and deuterium background correction systems. The measurement mode is dependant on the type of atomization tube used.

Table 1. Instrument Parameters

Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	*
Lamp current (ma)	5.0
Slit width (Nm)	0.5
Slit height	Normal
Wavelength (nm)	283.3
Sampler introduction	Sampler premix
Time constant (sec)	0.05
Replicates	2
Background correction	On

Peak height was used for wall atomization and peak area was used for platform atomization

Figure 1 shows signal traces for an aqueous standard and a human blood control. The forked platform tube was used. As stated previously, the platform temperature lags behind the tube temperature. This causes the analyte peak to come off later in the atomize step. The analyte peak is typically broad. Using peak area as the measurement mode compensates for the differences in analyte peak shape for the two matrices.

Figure 2 shows signal traces for an aqueous standard and a human blood control using a partition tube for wall atomization. The analyte peaks come off earlier in the atomize step. Even though the peaks do not come off at the same time, they have the same shape. The peak height is proportional to the concentration of Pb in this sample. With partition tube atomization, either measurement mode can be employed. Peak height generally gives higher absorbance values and was chosen over peak area in this case.

The furnace parameters for the SpectrAA-400Z are given in Table 2 for platform atomization and in table 3 for wall atomization. The programmed temperatures are normally higher for platform atomization than for wall atomization. The cyclic times reported are from sample injection to sample injection (1 replicate).

During the graphite furnace cool down following atomization, the sample dispenser retrieves the next sample.

Table 2. Forked Platform Tube - Zeeman, Cyclic Program Time ~93 Secs

Step no	Temp °C	Time (sec)	Gas flow (L/min)	Gas type	Read command
1	150	20.0	3.0	Normal	No
2	250	20.0	3.0	Normal	No
3	600	10.0	3.0	Normal	No
4	600	5.0	3.0	Normal	No
5	850	10.0	3.0	Normal	No
6	850	1.0	0.0	Normal	No
7	2300	1.5	0.0	Normal	Yes
8	2300	2.0	0.0	Normal	Yes
9	2500	6.0	3.0	Normal	No

Table 3. Partition Tube - Zeeman, Cyclic Program Time ~87 Secs

Step no	Temp °C	Time (sec)	Gas flow (L/min)	Gas type	Read command
1	95	20.0	3.0	Normal	No
2	130	20.0	3.0	Normal	No
3	350	5.0	3.0	Normal	No
4	350	10.0	3.0	Normal	No
5	800	5.0	3.0	Normal	No
6	800	1.0	0.0	Normal	No
7	2200	0.7	0.0	Normal	Yes
8	2200	2.0	0.0	Normal	Yes
9	2300	6.0	3.0	Normal	No

Table 4 gives the furnace parameters for the SpectrAA-400P for platform atomization. Table 5 gives the furnace parameters for wall atomization on the deuterium system.

Table 4. Forked Platform Tube - Deuterium, Cyclic Program Time ~90 Secs

Step no	Temp °C	Time (sec)	Gas flow (L/min)	Gas type	Read command
1	130	15.0	3.0	Normal	No
2	150	20.0	3.0	Normal	No
3	550	5.0	3.0	Normal	No
4	550	10.0	3.0	Normal	No
5	800	15.0	3.0	Normal	No
6	800	1.0	0.0	Normal	No
7	2200	1.3	0.0	Normal	Yes
8	2200	2.0	0.0	Normal	Yes
9	2400	3.0	3.0	Normal	No

Table 5. Partition Tube - Deuterium, Cyclic Program Time~90 Secs

Step no	Temp °C	Time (sec)	Gas flow (L/min)	Gas type	Read command
1	95	20.0	3.0	Normal	No
2	150	20.0	3.0	Normal	No
3	500	10.0	3.0	Normal	No
4	500	5.0	3.0	Normal	No
5	700	10.0	3.0	Normal	No
6	700	1.0	0.0	Normal	No
7	2100	0.8	0.0	Normal	Yes
8	2100	2.0	0.0	Normal	Yes
9	2400	3.0	3.0	Normal	No

Two replicate readings were taken for all standards and samples in this study. Solution volume injected was constant at 12 µL. The in run precisions were very good with %RSD values typically < 5%. Carbon buildup from the organic nature of the matrix did not occur over extended runs of greater than 30 blood samples.

The rinse solution for the sample dispenser was 10% isopropyl alcohol in D.I. water with 3 drops of 10% Triton X-100 added. The capillary remained clean inside and outside. Carryover problems were non-existent.

The 1:9 dilution of the blood samples with the working modifier reduces the background to a minimal level. Both Zeeman and deuterium background correction systems produced excellent accuracy and precision.

Forked Platform Tube Atomization

The absorbance values obtained with the Zeeman instrument were similar to those obtained with the deuterium instrument. In Figure 3 a calibration graph for the SpectrAA-400Z is shown. The calibration for the SpectrAA-400P is shown in Figure 4. These calibrations are with platform atomization measured in peak area.

The formula used to calculate the characteristic concentration (C.C.), that is, the concentration of analyte which will give an absorbance value of 0.0044, was [8]:

$$CC = \frac{(0.0044 \times C)_{(std)}}{A_{(std)}}$$

The middle standard, 30 µg/dL, is used in the calculation. For the Zeeman background correction system, the C.C. is equal to 1.4 µg/dL for a 12 µL sample (injection) volume. The deuterium system yielded a C.C. of 1.3 µg/dL for a 12 µL sample (injection) volume.

Blood controls used as samples represented the entire calibration range. Table 6 lists the results obtained with the Zeeman system. The found and %RSD values were calculated from data acquired over a three week period (n = 30). Similarly, Table 7 lists the results obtained with the deuterium system. The precision and accuracy are excellent for both systems using platform atomization.

Table 6. Blood Control Results, Zeeman-Platform

Control sample	True (µg/dL)	Found (µg/dL)	%RSD
Goat	43 ± 4	39 ± 0.70	1.8
Goat	15 ± 4	16 ± 0.15	0.9
Human	24 ± 4	23 ± 1.30	5.5
Human	5.0 ± 1	6.2 ± 0.20	3.2

Table 7. Blood Control Results, Deuterium-Platform

Control sample	True (µg/dL)	Found (µg/dL)	%RSD
Goat	43 ± 4	44 ±0.36	0.8
Goat	15 ± 4	15 ± 0.33	2.2
Human	24 ± 4	22 ± 0.70	3.2
Human	5.0 ± 1	5.3 ± 0.28	5.3

Partition Tube Atomization

Figure 5 shows a typical calibration curve for the SpectrAA-400Z with wall atomization. The absorbance values were similar to those for the SpectrAA-400P calibration in Figure 6. Since peak height was used, the absorbance values are greater than those in peak area. The higher values give lower, that is, better characteristic concentrations. For the Zeeman instrument, the C.C. is equal to 0.5 µg/dL for a 12 µL sample (injection) volume. The deuterium system gave a C.C. equal to 0.44 µg/dL for a 12 µL sample injection volume.

The results for the blood control samples obtained with the Zeeman system and wall atomization are recorded in Table 8.

Table 8. Blood Control Results, Zeeman–Partition

Control sample	True (µg/dL)	Found (µg/dL)	%RSD
Goat	43 ± 4	43 ± 0.51	1.2
Goat	15 ± 4	17 ± 0.31	1.9
Human	24 ± 4	24 ± 0.46	1.9
Human	5.0 ± 1	6.1 ± 0.30	4.9

The results for the deuterium system are summarized in Table 9.

Table 9. Blood Control Results, Deuterium – Partition

Control sample	True (µg/dL)	Found (µg/dL)	%RSD
Goat	43 ± 4	43 ± 0.58	1.3
Goat	15 ± 4	17 ± 0.09	0.5
Human	24 ± 4	22 ± 0.19	0.9
Human	5.0 ± 1	5.9 ± 0.28	4.8

Again, values were calculated from data acquired over a three week period (n = 30). The precision and accuracy are excellent for both systems using wall atomization.

Quality Control

Quality assurance and control are important factors in any application. Agilent QCP-3 software can automate the quality control procedures for blood lead determinations. Figure 7 illustrates the calibration with aqueous standards. Immediately following the calibration, an aqueous calibration verification standard and calibration blank are run as shown in Figure 8. The aqueous control standard was prepared from a different stock lead standard than that of the calibration standards. A low blood control was run as the first sample. Next, a continuing verification standard (high blood control) was run followed by a blank. The run continues with blood samples as shown in Figure 9.

The rate of continuing calibration verification with a blood control can be programmed by number of injections. Limit restrictions can be placed on the value obtained for the control standard along with specific corrective actions if the control falls outside the limits. This feature permits unattended operation and enhanced productivity.

Conclusion

Table 10 summarizes the data obtained in this study. The addition of control limits for the determination of lead in blood (from the New York State Department of Health method) shows that all data obtained is valid. The purpose of this study was to evaluate the use of both Zeeman and deuterium background correction systems for this application. The found values for both systems are all well within the specified limits. Evaluation of platform atomization and wall atomization showed that either atomization system gives accurate results. Long term precisions for both correction and atomization systems were excellent.

Either Zeeman or deuterium background correction in conjunction with platform or wall atomization can be used for the accurate, precise determination of lead in blood.

References

Table 10. Result Summary

Control Sample	True (µg/dL)	Zeeman platform	Partition	Deuterium platform	Partition
Goat	43 ± 4	39 ± 0.70	44 ± 0.51	44 ± 0.36	43 ± 0.58
Goat	15 ± 4	16 ± 0.15	17 ± 0.31	15 ± 0.33	17 ± 0.09
Human	24 ± 4	23 ± 1.3	24 ± 0.46	22 ± 0.70	22 ± 0.19
Human	5.0 ± 1	6.2 ± 0.20	6.1 ± 0.30	5.3 ± 0.28	5.9 ± 0.28

All values < 40 µg/dL are in range by within approximately 4 µg/dL
All values > 40 µg/dL are in range by within approximately 10%

1. "Preventing Lead Poisoning in Young Children", A Statement by the Centers for Disease Control, Atlanta, GA (October **1991**).
2. B. J. Stevens, J. B. Sanders, and R. Stux, "Lead Determination in Blood and Urine by Atomic Absorption", Varian Techtron (**1972**).
3. J. P. Matousek, and B. J. Stevens, "Biological Applications of the Carbon Rod Atomizer in Atomic Absorption Spectroscopy", Clin. Chemistry, **17**, 363 (**1971**).
4. H. Willard, L. Merritt, J. Dean, and F. Settle, "Instrumental Methods of Analysis", 6th Ed. (**1981**).
5. "Analytical Methods for Graphite Tube Atomizers", Varian Australia, Publication No. 85-100848-00 (September **1988**).
6. "GFAA, Chemical Modification and the EPA", Varian Spring Atomic Absorption Workshop (**1989**).
7. "Blood Lead Determination by Electrothermal Atomization Atomic Absorption Spectrometry", New York State Department of Health, Albany, NY (February **1991**).
8. "Detection Limit and Characteristic Concentration - What Do They Really Mean?", Varian Spring Atomic Spectroscopy Workshop (**1992**).

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem

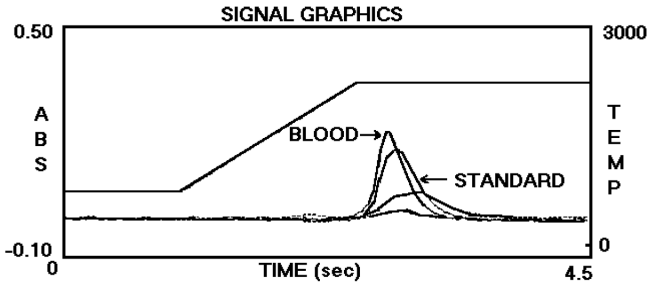


Figure 1. Signal Graphics using platform atomization.

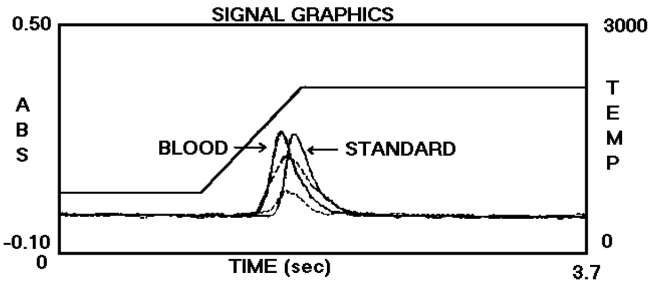
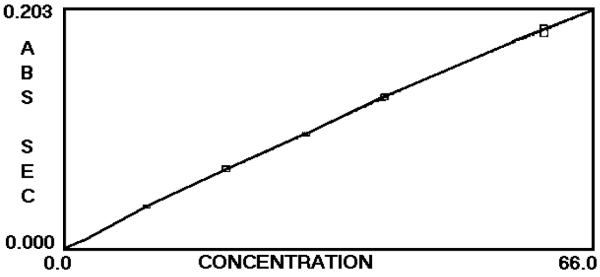


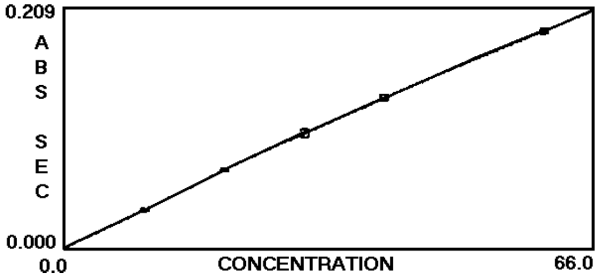
Figure 2. Signal Graphics using wall atomization.



Sample	Conc µg/dL	%RSD	Mean Abs	Readings	
Blank	0.0		-0.006	-0.007	-0.006
Standard 1	10.0	0.0	0.036	0.036	0.036
Standard 2	20.0	1.7	0.066	0.066	0.067
Standard 3	30.0	0.2	0.096	0.096	0.096
Standard 4	40.0	0.9	0.128	0.128	0.127
Standard 5	60.0	1.6	0.184	0.182	0.187

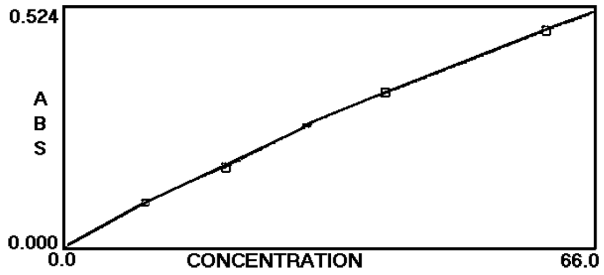
Conc **Mean**

Figure 3. Zeeman calibration using platform atomization.



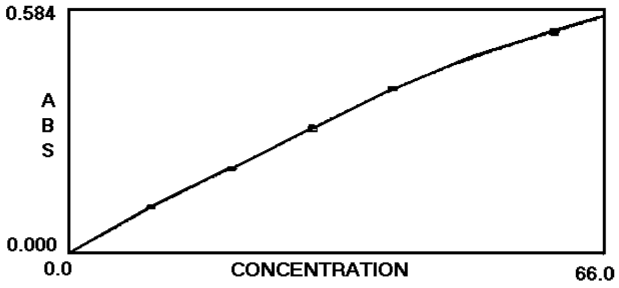
Sample	Conc µg/dL	%RSD	Mean Abs	Readings	
Blank	0.0		0.005	0.005	0.005
Standard 1	10.0	1.4	0.033	0.033	0.033
Standard 2	20.0	0.1	0.068	0.068	0.068
Standard 3	30.0	2.5	0.100	0.102	0.098
Standard 4	40.0	1.3	0.131	0.132	0.130
Standard 5	60.0	0.7	0.190	0.191	0.189

Figure 4. Deuterium calibration using platform atomization.



Sample	Conc µg/dL	%RSD	Mean Abs	Readings	
Blank	0.0		0.007	0.008	0.006
Standard 1	10.0	3.0	0.096	0.094	0.098
Standard 2	20.0	2.8	0.174	0.170	0.177
Standard 3	30.0	0.3	0.266	0.266	0.265
Standard 4	40.0	1.6	0.338	0.334	0.342
Standard 5	60.0	1.4	0.477	0.472	0.481

Figure 5. Zeeman calibration using wall atomization.



Sample	Conc µg/dL	%RSD	Mean Abs	Readings	
Blank	0.0		0.004	0.005	0.003
Standard 1	10.0	1.4	0.107	0.108	0.106
Standard 2	20.0	0.1	0.201	0.201	0.201
Standard 3	30.0	0.7	0.298	0.297	0.300
Standard 4	40.0	0.2	0.391	0.392	0.391
Standard 5	60.0	0.4	0.531	0.529	0.532

Figure 6. Deuterium calibration using wall atomization.

QC Protocol Report

OPERATOR cmf
DATE 6-15-92 PROGRAM 17 Pb blood
BATCH Blood Pb

Sample	Conc µg/dL	%RSD	Mean Abs	Readings	
Blank QC	0.0		-0.002	-0.002 15June92	-0.003 14:29
Standard 1 QC	10.0	6.0	0.035	0.036 15June92	0.033 14:32
Standard 2 QC	20.0	1.8	0.064	0.065 15June92	0.063 14:36
Standard 3 QC	30.0	0.0	0.091	0.091 15June92	0.091 14:39
Standard 4 QC	40.0	0.8	0.119	0.118 15June92	0.120 14:42
Standard 5 QC	60.0	0.2	0.170	0.170 15June92	0.170 14:46

Figure 7. Quality control 1.

Sample	Conc µg/dL	%RSD	Mean Abs	Readings	
QC Standard QC	43.7	0.4	0.129	0.130 15June92 %R =	0.129 14:50 101.7
QC Blank QC	0.0	33.0	0.002	0.002 15June92 ABS =	0.001 14:54 0.001
Sample 1 QC	17.4	0.4	0.057	0.057 15June92	0.057 14:57
QC Standard QC	44.5	0.1	0.131	0.131 15June92 %R =	0.131 15:00 103.4
QC Blank QC	0.0	46.0	0.001	0.001 15June92 ABS =	0.002 15:04 0.000
Sample 2 QC	15.0	0.6	0.050	0.050 15June92	0.050 15:07

Figure 8. Quality control 2

Sample	Conc µg/dL	%RSD	Mean Abs	Readings	
Sample 3 QC	44.7	0.2	0.132	0.132 15June92	0.132 15:11
Sample 4 QC	17.8	1.7	0.058	0.059 15June92	0.057 15:14
Sample 5 QC	15.6	1.3	0.050	0.050 15June92	0.049 15:17
QC Standard QC	44.5	0.2	0.131	0.132 15June92 %R =	0.131 15:21 103.5
QC Blank QC	0.0	65.6	0.001	0.001 15June92 ABS =	0.002 15:24 0.000
Sample 6 QC	14.4	0.2	0.048	0.048 15June92	0.048 15:28

Figure 9. Quality Control 3.

www.agilent.com/chem

Printed in the USA
November 1, 2010
AA110

Agilent Technologies