Method Guide: 40185

Atomic Absorption Full Method Pb in Whole Blood

Key Words

- Lead
- Blood
- QuadLine Background Correction
- Graphite Furnace
- Atomic Absorption
- Matrix Modification

Introduction

Lead is a non-essential element and toxic effects resulting from its extensive use have been known for many hundreds of years. Through the introduction of stringent safety precautions in industry, and regulations limiting its use, the number of cases of severe inorganic lead poisoning has fallen dramatically. Instances of toxicity, both industrial and non-occupational, do however, still occur, with symptoms of abdominal pain, fatigue, weakness, anaemia and peripheral neuropathy. In children, severe effects are more common, possibly resulting in CNS failure and death(1).

The concentration of lead in blood considered to be harmful to young children is 25 µg/dL. However, in the United States, this level has recently been lowered to 10 µg/dL(2).

Several methods for the determination of blood lead have been published over the last decade, with Graphite Furnace Atomic Absorption Spectrometry being the preferred technique.

This method describes two procedures for the determination of blood lead levels using Graphite Furnace Atomic Absorption Spectrometry; one using direct aqueous calibration and one using matrix matched standards.

Analytical range

Methods for the direct determination of lead in whole human blood are presented. The 3 sigma method detection limit for the aqueous method and the matrix matched standard method are approximately 0.78 µg/L and 0.61 µg/L respectively in the whole blood

Principle

Lead is determined directly in whole blood using Graphite Furnace Atomic Absorption Spectrometry. QuadLine background correction is used throughout. Ammonium phosphate, Triton X-100 and nitric acid are used as mixed matrix modifiers. Calibration can be performed either by using matrix matched blood standards or directly by using aqueous standards, with no observable loss in accuracy.

Methods

Reagents:

Nitric acid (Spectrosol grade).
Lead master standard (1000mg/L Spectrosol or equivalent).
Ammonium dihydrogen phosphate (AnalaR reagent).
Triton X-100 (AnalaR grade or equivalent).
Methanol (AnalaR grade or equivalent).
Phenol (AnalaR grade or equivalent).

All reagent examples available from:

Fisher Scientific
Bishop Meadow Rd
Loughborough,
LE11 5RG
UK.

Reference blood samples were obtained from:

N.I.S.T
Gaithersberg
MD 20899
USA

BioRad
3726 E. Miraloma Ave
Anaheim
CA 92806 USA

Sample collection

Blood samples were prepared in acid washed autosampler cups immediately before analysis.
A mixed matrix modifier solution containing 0.1 % v/v nitric acid, 0.2 % m/v ammonium dihydrogen phosphate and 0.5 % m/v Triton X-100 was used throughout

Aqueous calibration method

100 µL portions of whole blood were mixed with 900 µL of the mixed matrix modifier solution. Working standards were prepared daily by serial dilution of a master standard with 0.1 % v/v nitric acid.

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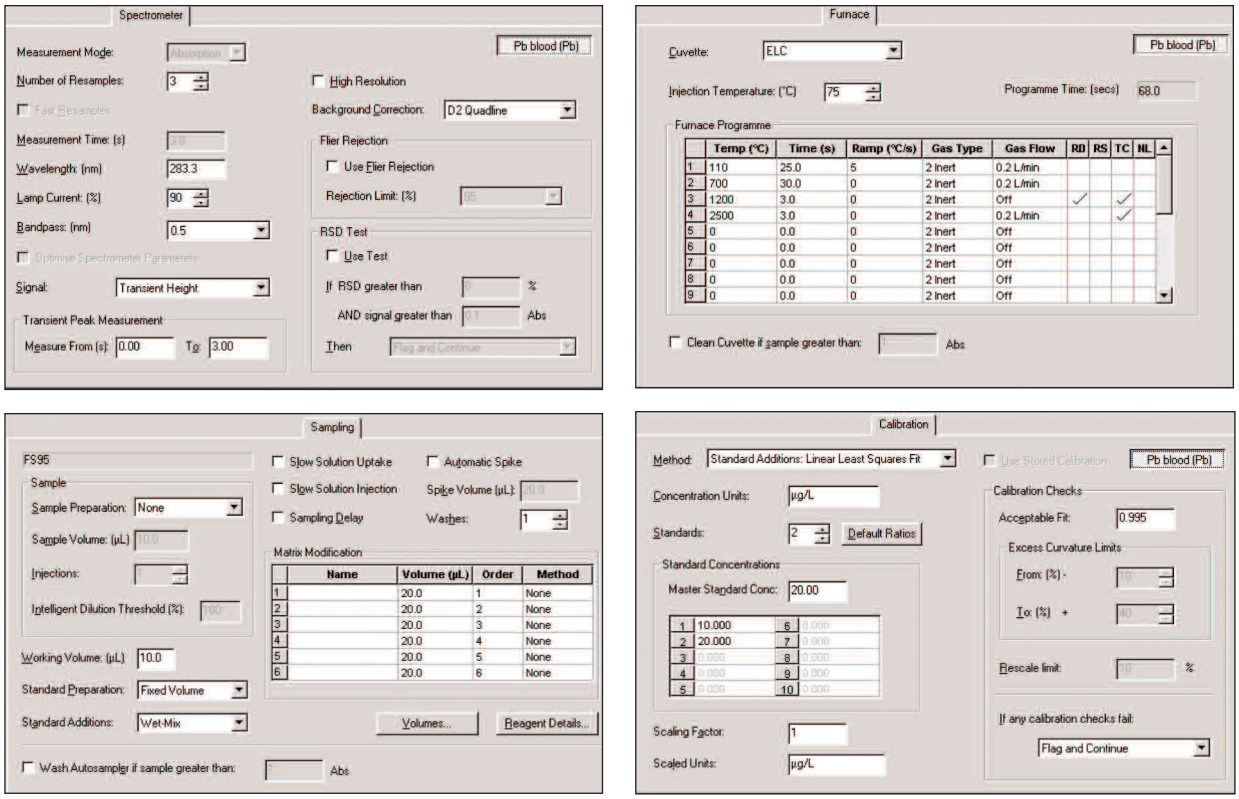

Figure 1: Analysis parameters

Method development

The mixed matrix modifier solution stabilised the lead to 900°C (figure 2), and resulted in clean, well shaped lead peaks when QuadLine background correction was used (figure 3). The alternative lead resonance line of 283.3 nm was used to overcome any possible phosphate interference at the primary lead resonance line of 217.0 nm; default parameters were used for most other instrumental conditions (figure 1).

Ridged, extended lifetime cuvettes (ELC's) were used throughout the work. An injection temperature of 75°C was used to reduce the furnace dry phase time and thus reduce the total furnace program time to a little over 1 minute. Peak height measurements were used exclusively.

It was observed that a build up of material on the autosampler tip occured after many analyses, causing inconsistent injections. This was eliminated by the use of 0.5 % v/v methanol, 0.1 % m/v Triton X-100 and 0.1 % v/v nitric acid in the autosampler wash fluid. Although dependent on environmental conditions, it is possible when analysing biological samples that microbial activity can also take place in and around the autosampler tip.

This problem can be solved by adding approximately 3 drops of phenol to the autosampler wash fluid.

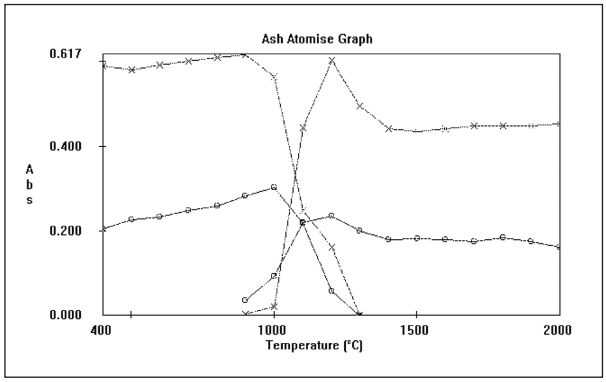

Figure 2: Ash/Atomise plot with mixed modifiers

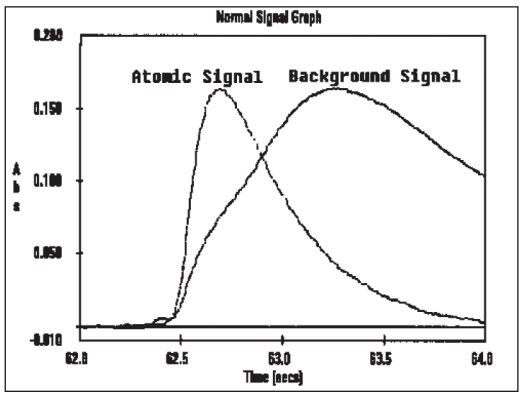

Figure 3: Pb signal from blood sample with optimised furnace program and mixed modifiers

Method validation

Both methods of standardisation were evaluated for accuracy and precision.

A series of spiking experiments were undertaken, and the gradients of the calibration lines were compared (figure 4, table 1).

Three blood samples were then spiked with known lead levels and analysed by both methods. The results are shown in table 2.

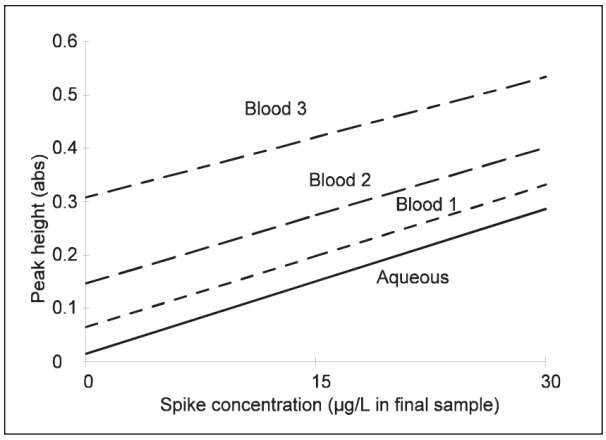

Figure 4: Spike experiment results

Sample	Gradient
Aqueous	0.0090
Blood 1	0.0089
Blood 2	0.0085
Blood 3	0.0076

Table 1: Spiking experiment results

Sample	Blood 1	Blood 2	Blood 3
Pb level (µg/L)	5.01	13.53	30.63
Pb added (µg/L)	12.00	12.00	12.00
Pb expected (µg/L)	17.01	25.53	42.63
Pb found (µg/L)	17.49	26.06	43.35
Recovery	102 %	102 %	101 %

Table 2: Recovery experiment results

Sample	Mean height (Abs)	S.D (Abs)	R.S.D
High blood	0.483	0.0059	1.2 %
Medium blood	0.316	0.0056	1.8 %
Low blood	0.209	0.0032	1.6 %

Table 3: Replicate experiment results - aqueous calibration

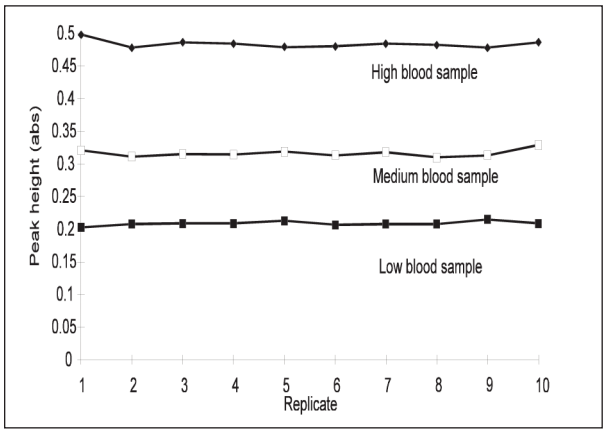

Figure 5: Validation experiment results

Sample	Mean height (Abs)	S.D (Abs Ht)	R.S.D
High blood	0.241	0.001	0.4 %
Low blood	0.074	0.003	4.2 %

Table 4: Replicate experiment results - matrix matched calibration

Reference material	Certified value (µg/L)	Method value (µg/L)
NIST SRM 955a-1	5.01±0.09	5.02±0.33
NIST SRM 955a-2	13.53±0.13	13.43±0.74
NIST SRM 955a-3	30.63±0.32	30.33±0.72
NIST SRM 955a-4	54.43±0.38	54.58±1.57

Table 5: Reference experiment results - aqueous calibration

Ten replicate analyses of three blood samples containing different lead levels were made in a single run using both methods of calibration. The results are shown in figure 5, tables 3 and 4.

Certified reference blood samples from a range of sources were analysed by both methods, and the results are shown in tables 5 and 6.

Reference material	Certified value (µg/L)	Method value (µg/L)
BioRad 63801	5.2±1.1	5.37±0.27
BioRad 63802	24.5±3.7	23.46±0.48

Table 6: Reference experiment results - matrix matched

Results

For the aqueous method, the spike experiment results show that the gradient of the calibration lines are similar between the aqueous and blood matrices. The differences between the aqueous standards and blood samples are relatively minor indicating insignificant interference. In this situation, the direct method of aqueous calibration can be used successfully. The results of the recovery experiments in table 2 confirm this, with full recovery of the spike obtained for all samples.

Consistent results were obtained throughout the work, shown in table 3 and figure 5. Particularly, no trace of any carbonaceous material was observed inside the cuvette after atomisation. The use of a fast ash ramp is thought to reduce carbon build-up by releasing smoke which is removed by the internal gas flow.

The absence of this build up of material, and with the use of an ELC cuvette, allowed several hundred samples to be analysed without any intervention.

The characteristic mass (mass of analyte required to generate a signal peak of 0.0044 absorbance units high, or 0.0044 abs.s in area) for both methods, calculated from the spike experiment results, was 3.6 pg in peak height and 8.8 pg in peak area for the aqueous method, and 3.7 pg in peak height and 7.1 pg in peak area for the blood matrix method. The 3 sigma method detection limit, calculated from the low blood sample results, using both aqueous and matrix matched methods was 0.78 µg/L and 0.61 µg/L respectively in the original blood samples.

Conclusions

Methods for the determination of lead in whole blood samples by Graphite Furnace Atomic Absorption Spectrometry have been developed.

Hot injections and a fast furnace program, with the use of an ELC cuvette, allow several hundred samples to be analysed directly with no problems.

Both methods of standardisation presented are suitable for this application, giving accurate results and allowing long unattended analyses to be performed.

References

(1) Walker, A., Trace Element Analysis, SAS, 1987, 10

(2) Centres for Disease Control, Preventing Lead Poisoning in Young Children. U.S Dept of Health and Human Services (Report), Atlanta, GA.1991.

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