Method Guide: 40714

Full Method - Pb in Whole Blood using Omega Platform Cuvettes

Key Words

- Quadline D_2 Background Correction
- Atomic Absorption
- Blood
- Graphite Furnace
- Lead
- Matrix Modification
- Omega Platform Cuvette

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 the procedure for the determination of blood lead levels using Graphite Furnace Atomic Absorption Spectrometry using mixed matrix modified matched calibration standards and Omega platform cuvettes.

Analytical range

Methods for the direct determination of lead in whole human blood are presented. The 3s method detection limit for the calibration method is 0.42 µg/L in the whole blood and the characteristic mass is 5.1 pg.

Principle

Lead is determined directly in whole blood using Graphite Furnace Atomic Absorption Spectrometry with Omega platform cuvettes. Quadline D_2 background correction is used throughout. Ammonium phosphate, triton X-100 and nitric acid are used as mixed matrix modifiers. Calibration can be performed directly using aqueous Pb standards diluted with the mixed matrix modifier. The use of a furnace vision system,such as the Thermo Scientific GFTV, allows events inside the cuvette to be visualized and is an essential tool when performing this analysis.

Reagents and Samples

Reagents:

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

All available from:

Fisher Scientific
Bishop Meadow Rd
Loughborough
LE11 5RG

Samples

Reference whole blood certified reference materials and horse blood samples were obtained from:

LGC Promochem
Queens Rd
Teddington
Middlesex
TW11 0LY

and

TCS Biosciences Ltd
Botolph Claydon
Buckingham
MK18 2LR

Sample Preparation

Whole 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 ($[NH_3]_2HPO_4$) and 0.5 % m/v Triton X-100 was used. 100 µL portions of whole blood were mixed with 900 µL of the mixed matrix modifier solution directly in the autosampler vials.

Method development

Calibration method

Earlier research (Thermo Fisher Scientific Application Note: Full method - Pb in whole blood), where spike experiments resulted in identical calibration curve gradients for aqueous standards and spiked bloods, demonstrated that differences between them were relatively minor, indicating insignificant interferences. Therefore, working standards were prepared daily by serial dilution of an aqueous master standard using the mixed matrix modifier solution as the diluent. A wash solution comprising 0.1 % HNO_3 and 0.5 %m/v Triton X-100 was used.

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Method parameters

The primary wavelength for Pb is at 217.0 nm, however, the alternative lead resonance line of 283.3 nm was used to overcome any possible phosphate interference at the primary resonance line.

Use of both Quadline D_2 and Zeeman background correction was investigated, examination of peak profiles (Figure 1) demonstrated that there was no significant difference in the corrected signals obtained using either background correction method. On the basis of this finding Quadline D_2 correction was selected since it will always result in slightly improved sensitivity compared to a Zeeman background correction.

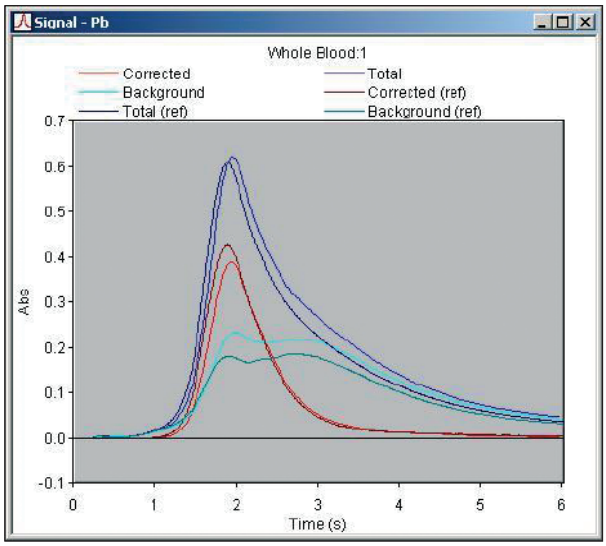


Figure 1: Profiles for Zeeman background correction compared with D_2 background correction (ref)

Cuvette Type

In analyses using off-the wall atomization (i.e. normal cuvettes) samples are vaporized from a hot cuvette wall into a cooler, vapour phase, which can favour the formation of analyte molecules and can lead to 'vapour phase interferences'. In a platform cuvette the platform is predominantly heated by radiation from the cuvette wall and consequently the temperature of the platform lags behind that of the cuvette wall. The result of this difference is that when the sample is eventually vaporized and atomised, it does so into a hotter vapour phase temperature, which reduces analyte molecular formation.

The Omega cuvettes used in this study have an integrated platform (Figure 2) and take advantage of the superior performance given by ELC (Extended Lifetime Cuvette) technology. The use of ELC technology ensures a cuvette/platform combination with considerably longer lifetimes than previously possible with typical thin pyrolytic coating products.

In many cases and definitely for the relatively volatile element Pb the use of a matrix modifier should be employed to further enhance the effect of the platform.

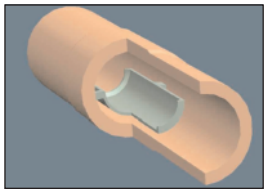


Figure 2: The Omega ELC Cuvette with integrated platform

Ammonium dihydrogen phosphate was selected as the matrix modifier as it is thought to have two modes of operation. It has been hypothesized (3) that the basic component of the molecule may aid volatilization of halides formed during the ash-programme and that the phosphate component of the molecule may help in stabilization of the analyte, which can help to delay the volatilisation of the analyte during the atomization phase.

GFTV was utilized throughout the method development procedure to observe the drying and ashing phases of the furnace programme. This visual monitoring enabled the injection parameters to be optimized and slight changes to be made to the default parameters suggested by the SOLAAR Software. Such changes are necessary due to the influence of sample matrix and can dramatically improve the smoothness of the drying and ash phases, reducing imprecision frequently observed if samples boil or spit in these early phases. Close visual monitoring also allows a more accurate assessment of the timings necessary to achieve the required effects and can enable analysis times to be reduced where appropriate, improving productivity. Figure 3 illustrates the important method parameters.

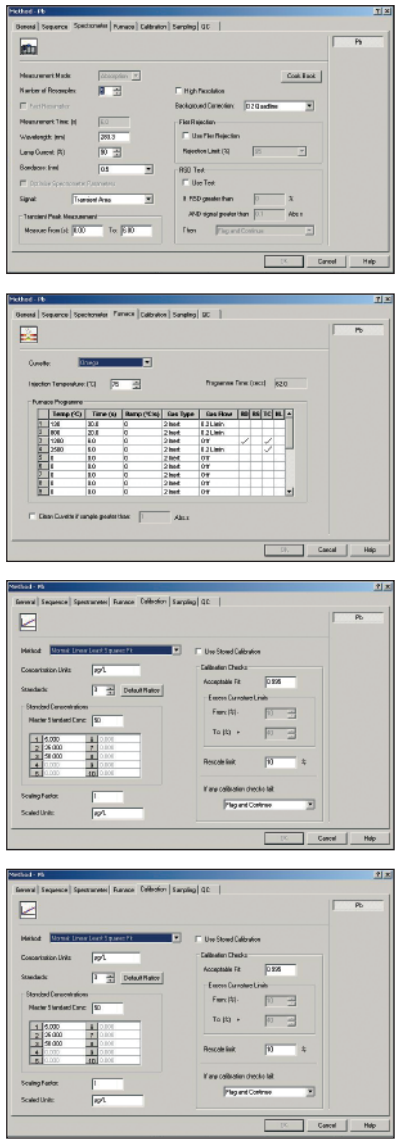
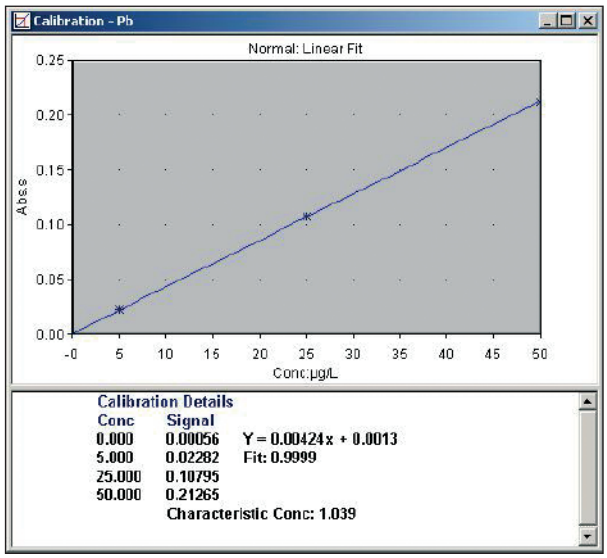


Figure 3: SOLAAR Software method parameters

Method validation

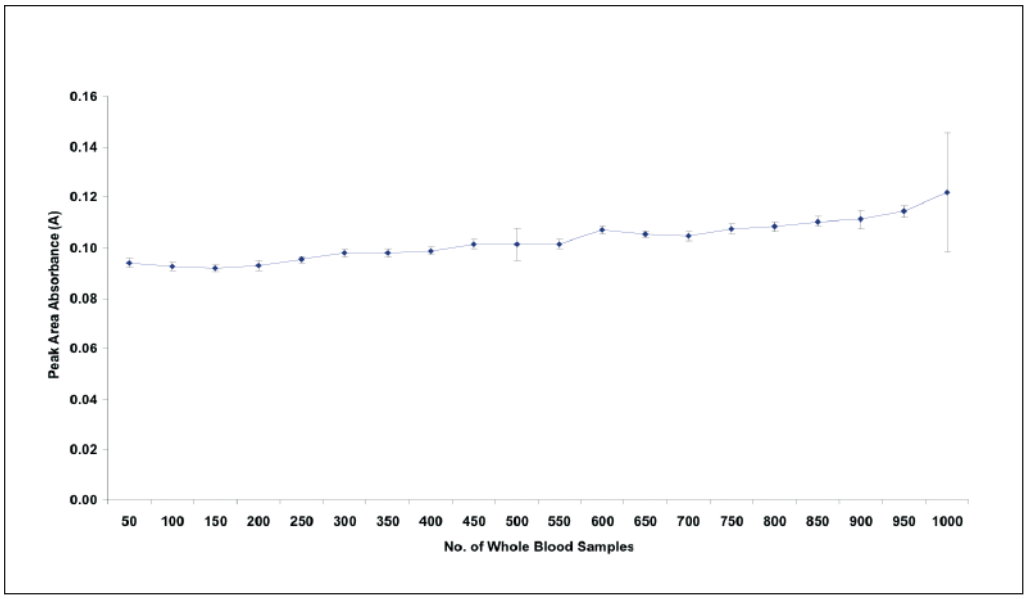
1000 samples of whole blood analysis - An analysis of whole blood was carried out to assess the stability of the method over the course of an extended (>24 hour) run and also to demonstrate the robustness of the Omega platform cuvettes. A whole horse blood sample stabilized in EDTA was spiked with 20 ppb Pb at the 1:10 dilution stage and 1000 firings (20 samples, 50 replicates of each) were analysed.

CRM analysis - Seronorm whole blood Level 2. (Batch No. 2903058) was reconstituted and diluted 1:10 with the mixed matrix modifier solution. A 50 µg/L aqueous Pb standard was prepared using the mixed matrix modifier as the diluent. The SOLAAR software was set to perform fixed volume, automatic dilutions of the master standard to generate a 3-point calibration at 5 ppb, 25 ppb and 50 ppb. 5 replicate analyses of the whole blood CRM were carried out.



Results

1000 samples of whole blood analysis - The mean %RSD for each batch of 50 replicates is <1.5 % (Figure 4) and only degrades for the last whole blood sample, which is most likely to be due to the sample being sat in the autosampler vial for such a long period of time (~28 hours). It is this factor that also causes the signal to consistently drift upwards. This is due to sample diluent evaporating, causing the blood samples to become slightly more concentrated as the analysis is performed. This apparent drift could be eliminated by reducing the amount of time samples wait in the autosampler prior to analysis.



CRM analysis - The calibration curve generated is shown in figure 5 and shows the excellent linearity obtained. The characteristic mass for this analysis was 6.2 pg using peak area measurements. The mean measured Pb content of the 5 replicate whole blood samples = 380 µg/L, a recovery of 99.3 % of the certified value, and squarely within the analytical range of the CRM which is 361-396 µg/L and the %RSD of the 5 replicate samples = 0.7 %.

Conclusions

A method for the determination of lead in whole blood samples by Graphite Furnace Atomic Absorption Spectrometry using Omega platform cuvettes has been developed. Use of a hot injection and a relatively fast furnace program combined with the use of an Omega platform cuvette and a mixed matrix modifier allow 1000 whole blood samples to be analyzed directly with no problems. This method demonstrates that blood lead analysis can successfully be carried out using Omega platform cuvettes in addition to normal cuvette designs, 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

3) Brown A.A. and Dymott T.C. The use of Platform Atomisation and Matrix modification as methods of interference control in Graphite Furnace analysis. Pye Unicam Ltd.

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Africa
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Austria
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