AAS
APPLICATION NOTES

The determination of lead in human blood using porcine blood standards

GBC

Introduction

Accuracy in the determination of blood lead is essential in screening for childhood lead poisoning, occupational exposure monitoring and population surveys. Due to the difficult matrix and the low levels of lead in blood (0.05 – 10 µmol/L or 0.01 – 2 mg/L) direct flame atomic absorption is not satisfactory. Methods involving solvent extraction and flame AAS (AS 2411)[1] can give accurate results but these methods involve a number of manipulation steps and require at least 3 mL of blood for a single determination. Hence these methods are slow and cannot be used for screening programmes where small volumes (<1 mL) of capillary blood are collected. This report describes a rapid automated method for the determination of lead in blood that reduces the potential for contamination by reducing the number of manipulations.

Sample collection

Avoiding contamination is of primary importance in the collection of samples for the determination of blood lead[2]. Samples should be collected according to the procedures described in AS2636[3]. Particular care should be taken to check all sampling equipment according to Appendix A of this standard and to ensure that all steps for cleaning the sampling site are followed.

Instrumentation

The GBC automated graphite furnace system and GBC atomic absorption spectrophotometer were used for this analysis. The GBC graphite furnace system comprises the graphite furnace (GF) and programmable automatic sample loader (PAL), which will accommodate auto standard preparation, auto matrix modification and auto recalibration of the curve. The GBC double beam atomic absorption spectrophotometer offers complete report facilities and high speed HYPER-PULSE background correction for accurate correction of the blood matrix.

The 283.3 nm lead line was used in preference to the 217.0 nm line. Although the sensitivity at 283.3 nm was only half that at 217.0 nm, the noise was lower, the calibration curve more linear and the background absorbance was lower. Due to the volatility of lead, a total pyrolytic graphite platform was used throughout this analysis. The platform allows for atomisation into a heated atmosphere, preventing recombination of molecules.

Procedure

Sample preparation

Blood samples were diluted (1 + 5) with a solution of Triton X100 (0.5 mL/L) and diammonium hydrogen phosphate (5 g/L). In the determination of lead in blood each manipulation of the sample increases the chance of contamination from dust, atmospheric lead, reagents and containers. To minimise the potential for these errors, dilution of the sample was carried out in the sample container (Beckton and Dickinson – Microtainer capillary whole blood collector, 300 µL fill line containing 0.39 mg of disodium EDTA). The PAL autosampler was modified to allow these tubes to fit directly into the sampler tray. The normal PAL sample vials are 25 mm high and 12 mm in diameter. The Microtainer vials are 45 mm high and 6 mm in diameter. Teflon inserts were fitted to the standard PAL carousel to allow direct sampling from the Microtainer vials.

Calibration

Standard blood solutions were prepared by spiking porcine blood containing 1.3 mg/mL of disodium EDTA with aqueous lead nitrate solution to give six standards covering the range 0.6 – 4.6 µmol/L. The lead content of these standards was determined by AS2411. Calibration standards for the GBC graphite furnace system were prepared by diluting the blood standard solutions (1 + 5) with Triton X100 (0.5 mL/L) and diammonium hydrogen phosphate (5 g/L) in Beckton and Dickinson Microtainers containing no EDTA.

GBC atomic absorption spectrophotometer and graphite furnace system parameters

A complete printout of the operating parameters for the spectrophotometer, furnace and autosampler is shown in Figure 1. The standard concentrations listed are in µmol/L.

Operating Parameters	
Element	Pb
Wavelength	283.3 nm
Slit Width	1.0 nm
Atomization	Furnace
Lamp Current	8.0 mA
EHT (gain)	– 284 V
Scale Expansion	1.000
Integration Time	0.3 s
Beam Mode	Double Beam
Instrument Mode	Background Correction

Graphite Furnace Parameters						
Step No.	**Final Temp °C**	**Ramp Time (sec)**	**Hold Time (sec)**	**Inert Gas**	**Aux. Gas**	**Read On**
1	150	20.0	20.0	No	Yes	No
2	250	15.0	5.0	No	Yes	No
3	800	20.0	20.0	No	Yes	No
4	800	1.0	1.0	No	No	No
5	2400	2.0	2.0	No	No	Yes
6	2500	1.0	1.0	No	Yes	No

Autosampler Details	
Sample Volume (µL)	10
Modifier Volume (µL)	0
Number of multiple injections	1
Number of sample repeats	2
Dry steps for multiple injections	1
Inject on step number	1

Standards Details	
Number of standards	6
Standard 1 concentration	0.66
Standard 2 concentration	1.21
Standard 3 concentration	1.72
Standard 4 concentration	2.94
Standard 5 concentration	3.67
Standard 6 concentration	4.58
Recalibration rate	0

Figure 1: Operating parameters for GBC automated graphite furnace system and atomic absorption spectrophotometer

Potential problems

Several potential problems related to this application were addressed individually. The solutions to these problems are outlined below.

1. Injection error. Injection onto the graphite platform must be carefully aligned. Misalignment can cause:
   a) Spilling of solution onto the furnace wall. This can be detected in the peak height plot, where two peaks can be seen. One is due to atomisation from the wall and one is due to the delayed atomisation from the platform.
   b) Lifting of the platform. Adhesion of the capillary to the platform can cause lifting of the platform on removal of the capillary from the tube.
   c) High injection. This will not allow contact of blood to platform. Subsequently, the blood will transfer to the injection port of the tube on retraction of the capillary. Correct probe set-up, as illustrated in Figure 2, will eliminate these problems.

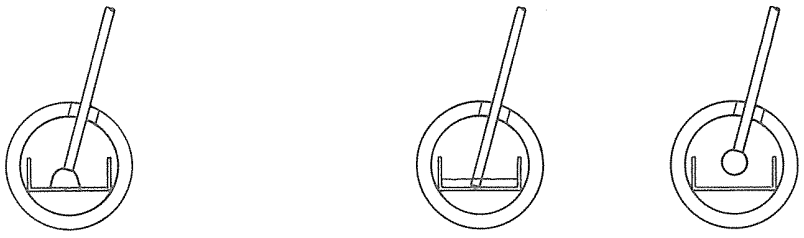

Figure 2: Correct injection procedure for platform

2. Poor reproducibility. This is usually associated with erratic drying of the sample. Method development should include studying the sample during the dry and ash stages by using a mirror held at the right-hand side of the workhead. If any violent reaction occurs, the temperature should be reduced or the ramp time increased.

3. Peak splitting. Prior to establishing the existing method, some errors were noticed due to split peaks. This occurs due to the existence of two or more compounds of lead. The addition of diammonium hydrogen phosphate ensures that all lead will exist as a single compound.

4. Memory effects. To assist with the reduction of sample carryover, 0.01% Triton X100 and 0.1% nitric acid can be added to the rinse solution.

Results

An example of a typical calibration is shown in Figure 3.

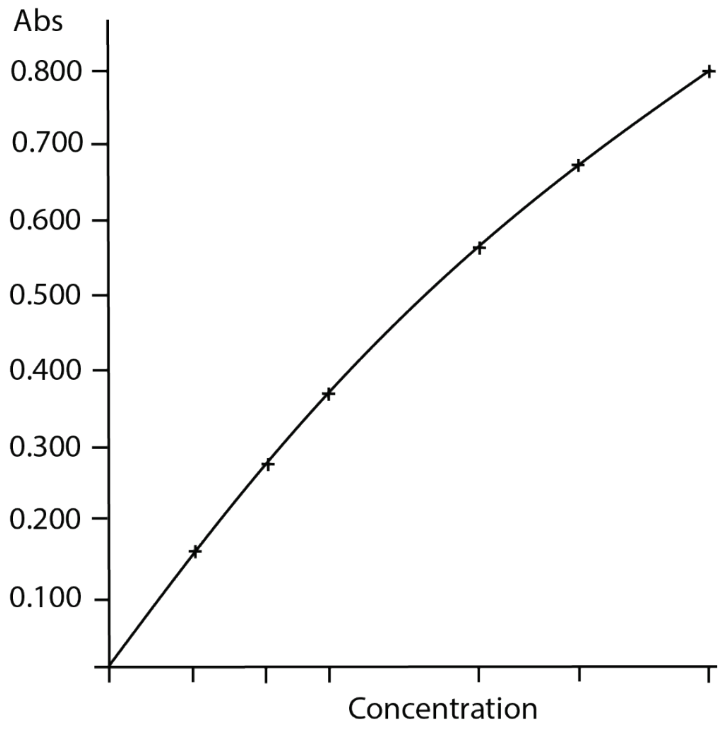

Figure 3: Calibration curve for lead in blood

The method gives excellent precision. Printouts of twenty replicate measurements of a blank and a sample are shown in Figure 4. A relative standard deviation of 1.76% was obtained on a sample containing 1.32 µmol/L lead.

Reading 1	0.018	Reading 11	0.014
Reading 2	0.017	Reading 12	0.014
Reading 3	0.017	Reading 13	0.012
Reading 4	0.015	Reading 14	0.014
Reading 5	0.017	Reading 15	0.014
Reading 6	0.014	Reading 16	0.015
Reading 7	0.014	Reading 17	0.013
Reading 8	0.013	Reading 18	0.018
Reading 9	0.016	Reading 19	0.014
Reading 10	0.015	Reading 20	0.013

Blank: Mean of 20 readings = 0.015

Reading 1	0.359	Reading 11	0.356
Reading 2	0.339	Reading 12	0.360
Reading 3	0.358	Reading 13	0.349
Reading 4	0.357	Reading 14	0.347
Reading 5	0.353	Reading 15	0.348
Reading 6	0.347	Reading 16	0.354
Reading 7	0.356	Reading 17	0.345
Reading 8	0.358	Reading 18	0.355
Reading 9	0.342	Reading 19	0.353
Reading 10	0.351	Reading 20	0.344

Standard 1: Mean of 20 readings = 0.351 R.S.D. = 1.76%

Figure 4: Precision for replicates of blank and blood sample containing 1.32 µmol/L lead

The accuracy of the method was assessed by analysing samples distributed by the External Quality Assurance Scheme, Birmingham, U.K.[4] Results obtained on these samples are shown in Table 1.

EQAS				
Specimen No.	**No. Labs**	**Mean**	**Standard Deviation**	**This Method**
300	64	2.17	0.13	2.11
301	81	3.30	0.27	3.47
302	83	1.31	0.11	1.26
303	79	2.11	0.14	2.16
304	77	1.45	0.14	1.44
305	61	2.64	0.15	2.62
306	79	3.25	0.24	3.50

Table 1: (all results µmol/L)

Further validation of the method was obtained by analysing two Nycomed "Seronorm" standards[5] and the results are shown in Table 2.

Batch	Preliminary Recommended Values	This Method
902	0.3	0.3
901	1.6	1.7

Table 2: (all results µmol/L)

Conclusion

A successful method has been developed for the determination of the lead content in human blood. The method incorporates the use of special Microtainer cups to eliminate contamination and the use of a graphite platform to prevent vapour phase interferences in the analysis. In an effort to decrease sampling time over a standard additions method, porcine blood spiked with lead nitrate was used. These standards provided good replication of the matrix and an accurate calibration. The method was verified by running EQAS samples and Nycomed "Seronorm" standards. All experimental values were in excellent agreement with the certified values of these standards and reproducibility was in the range of 1–2% relative standard deviation.

References

1. Australian Standard 2411 (1980), Determination of Lead in Venous Blood (Flame Atomic Absorption Spectrometric Method).

2. Sinclair, D.F. and Dohnt, B.R., Sampling and Analysis Techniques Used in a Blood Lead Survey of 1241 Children in Port Pirie, South Australia, Clinical Chemistry 30, 1616 (1984).

3. Australian Standard 2636 (1988), Sampling of Venous and Capillary Blood for the Determination of Lead or Cadmium Concentration.

4. Bullock, D.G., Smith, N.J. and Whitehead, T.P., External Quality Assessment of Assays of Lead in Blood, Clinical Chemistry 32, 1884 (1986).

5. Seronorm™ Whole Blood, Batch nos. 901 & 902. Produced by: Nycomed AS Diagnostics, P.O. Box 4284, Torsnov, 0401 Oslo 4, Norway.

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