Sensitive, high-throughput analysis of lead in whole blood using the Agilent 7500cx ICP-MS with ISIS-DS

Application note

Clinical research

Authors

Amir Liba, Craig Jones and Steven Wilbur

Agilent Technologies
USA

Ryszard Gajek and Flavia Wong

State of California Department of Public Health, Richmond, CA, USA

Introduction

As a result of the potent toxicity of lead, emphasis has been placed on its analysis in biological fluids. For the analysis of Pb in whole blood, minimal sample handling is critical in order to minimize contamination. A highly robust and stable instrument is essential to minimize signal suppression and drift due to the complex sample matrix.

Furthermore, laboratories typically require the highest possible sample throughput in order to cope with large numbers of samples. Currently, many clinical research laboratories still use graphite furnace atomic absorption (GFAA), and anodic striping voltametry (ASV) for the analysis of lead in whole blood [1]. Although both techniques may achieve the required sensitivity (10 µg/dL), they are lacking in speed and ease-of-use.

ICP-MS, with discrete sampling is a simpler, faster analytical method, and better suited to this application. In addition to increasing sample throughput, the ISIS-DS reduces the total amount of sample matrix to which the ICP-MS interface is exposed. This provides improved long term stability with this

type of complex sample matrix. As a result, instrument maintenance is reduced, further increasing overall sample throughput. The ISIS-DS is fully integrated with the Agilent 7500 (and 7700) Series ICP-MS instruments and is controlled by the instrument's operating software.

Configuring the ISIS-DS is simple, since it consists essentially of a switching valve and sample loop. The ICP-MS is tuned for typical robust plasma conditions providing a highly reproducible and accurate analysis.

Experimental

Instrument parameters were optimized to normal robust plas- ma conditions with oxide levels ~1% (CeO^+/Ce^+) (Table 1).

Table 1. ICP-MS operating parameters

Forward power (W)	1550
Sample depth (mm)	8
Carrier gas (L/min)	0.85
Makeup gas (L/min)	0.15
Extract 1 (V)	0
ISIS loop length (cm)	50
ISIS loop id (mm)	0.8
ISIS loop volume (µL)	250
ISIS stabilization time (sec)	20

Samples were supplied by the California Department of Public Health (CADPH) and analyzed according to the CADPH method that specifies a 50x dilution of the whole blood. The high matrix tolerance of the Agilent 7500cx ICP-MS allows whole blood to be analyzed routinely at a 10x dilution and many labs take that approach. However, in compliance with the CADPH method, a 50x dilution was applied for this work.

The samples consisted of the following: base blood, 1 ppb spike base blood, 1 ppb CCV, CCB (diluent only), and the following CADPH Standard Reference Materials (SRM); low blood QC (4.98 ± 0.17 µg/dL Pb where 1 µg/dL = 10 ppb), medium blood QC (9.66 ± 0.12 µg/dL Pb), and high blood QC (19.03 ± 0.29 µg/dL Pb) samples. These samples were analyzed repeatedly for a total of approximately 300 analyses.

Calibration standards were not matrix-matched and consisted of a blank, 0.01, 0.05, 0.1, and 1 µg/dL Pb, yielding an instrument detection limit of 3.09 × 10–4 µg/dL (3.1 ppt) (Figure 1).

Calibration standards were prepared in an NH_4OH, EDTA, 1-butanol, Triton X-100 diluent (2% NH_4OH, 4% 1-butanol, 0.1% EDTA, 0.1% Triton X-100).

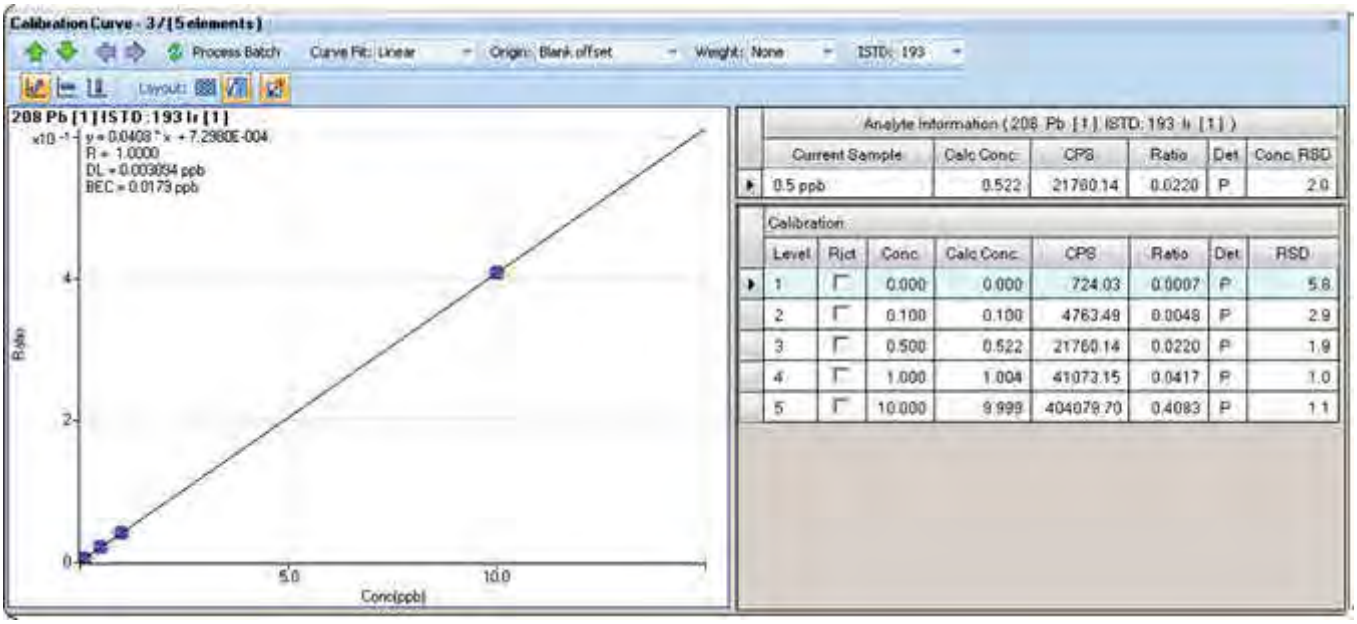

Figure 1. Calibration curve and table

Data and Results

Sensitivity and Precision

To determine the analytical method sensitivity and precision for Pb, seven replicates of the 0.01 µg/dL standard were acquired and the standard deviation was multiplied by 3.14 (99% confidence limits for student t-test) to give the measured on-instrument detection limit (DL). Table 2 shows the concentration and standard deviation (SD) used to calculate the resulting on-instrument detection limit of 5.4 x 10-4 µg/dL (5.4 ppt). In-sample method detection limits would require correction for the sample prep dilution factor, which in this case was 50x. However, Agilent standard procedure specifies 10x, which would result in a MDL of 54 ppt.

Table 2. Precision and Measured Detection Limits for Lead

Date	Time	Sample (µg/dL)	Measured Pb Concentration (ppb)	Measured Pb Concentration (µg/dL)
10/13/2009	12:24 PM	0.01	0.0997	0.00997
10/13/2009	12:24 PM	0.01	0.0985	0.00985
10/13/2009	12:25 PM	0.01	0.0968	0.00968
10/13/2009	12:26 PM	0.01	0.1001	0.01001
10/13/2009	12:27 PM	0.01	0.0985	0.00985
10/13/2009	12:29 PM	0.01	0.0952	0.00952
10/13/2009	12:30 PM	0.01	0.0972	0.00972
Standard Deviation			0.001734	0.0001734
On-instrument Detection Limit			5.445×10^{-3}	5.445×10^{-4}

Whole Blood Results

Three CADPH SRMs, spike base blood, and Continuing Calibration Verification/Blanks (CCV/CCB) were repeatedly analyzed, totaling 301 individual analyses. There were over 40 analyses per sample, with the exception of the CCV/CCB pair, which was analyzed after every ten analytical runs. The entire analysis took 259 minutes, resulting in a sample-to-sample run time of 52 seconds. Table 3 details the sample results.

Reference values for the SRM samples are listed in Table 4. Note that the sample concentration as injected into the ICP-MS ranged from approximately 0.099 to 0.381 µg/dL (~1-4 ppb), illustrating the ability of the Agilent 7500cx ICP-MS to accurately measure low analyte concentrations in a complex matrix.

Internal Standard (ISTD) Recoveries

The long term instrument stability can be demonstrated by monitoring ISTD recovery verses time. Figure 2 details the ISTD recoveries for the entire analytical run. Both ^{103}Rh and ^{193}IR are plotted here, though only ^{193}IR was used for all calculations. Control limits (dotted lines) were set at 85% to 105%. ISTD stability was excellent through the entire run with no significant drift observed. In addition, ISTD suppression due to the 50x whole blood matrix was minimal, demonstrating the robustness of the Agilent 7500cx ICP-MS. The slightly elevated points visible in the plot are due to the small increase in nebulization efficiency when the non-matrix matched QC samples (CCB and CCV) were measured.

Table 3. Results for Whole Blood Samples. All Samples Were Diluted 50x Except CCV/CCB.

Sample name	Sample number (n)	Ave Pb conc. (µg/dL)	Standard deviation	% RSD	% Recovery
Base Blood	52	0.004	0.0003	6.09	NA*
Base Blood Spike (1 ppb)	45	0.097	0.0011	1.20	97
CCB	26	0.0002	0.00010	46.5	NA*
CCV	26	0.099	0.0014	1.36	99
Low Blood SRM	45	4.911	0.0687	1.40	99
Medium Blood SRM	44	9.696	0.1136	1.18	100
High Blood SRM	44	18.947	0.2231	1.18	100

*NA-not applicable

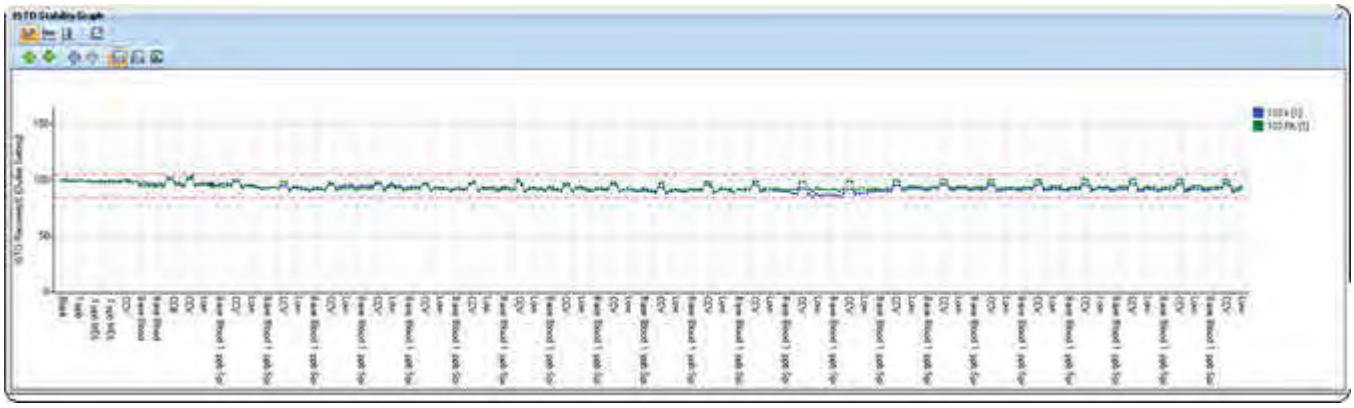

Figure 2. ISTD recoveries (due to space limitation, not every sample name is displayed on the X-axis).

Table 4. Reference Values for the CADPH Standard Reference Materials

SRM	Value (undiluted)	Value (50x dilution)
Low Blood SRM	4.98 ± 0.17 µg/dL	0.0996 µg/dL
Medium Blood SRM	9.66 ± 0.12 µg/dL	0.1932 µg/dL
High Blood SRM	19.03 ± 0.29 µg/dL	0.3806 µg/dL

Conclusions

Whole blood analysis presents several challenges for ICP-MS. Rapid sample handling, high sensitivity, excellent long term stability, and high tolerance to complex matrices are all critical to a successful analysis. The Agilent 7500cx ICP-MS with ISIS-DS allows for rapid (52 sec) sample- to-sample analyses with minimal to no carryover and superb sensitivity and long term stability throughout a sequence of more than 300 samples. The highly robust plasma of the Agilent 7500cx ICP-MS eliminates the need for matrix-matched standards and blanks, further simplifying the analysis.

References

1. C40-A Analytical Procedures for the Determination of Lead in Blood, 06/01/2001, Clinical and Laboratory Standards Institute (CLSI), US, www.clsi.org

www.agilent.com/chem

Published August 5 2015
Publication number: 5990-5416EN

Agilent Technologies