ICP-MS Determination of Lead Levels in Autopsy Liver Samples: An Application in Forensic Medicine

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INTRODUCTION

Air, water, and soil contamination are the basis of environmental problems. The fact that the environment is increasingly dangerous to human health due to pollution, urbanization, and especially industrial development has raised the contact with heavy metals not only for humans but all living things (1, 2). Lead (Pb) is an abundant and functional metal which has been utilized since ancient times. This non-essential element has been extensively dispersed into nature and human exposure to Pb has increased (3). However, in most countries, elimination of lead from water lines, food packaging and paint as well as the use of lead-free petroleum products has diminished exposure to lead in recent years (4). In 2006, the International Agency for Research on Cancer (IARC) has classified lead compounds and inorganic lead as "Group 2A" probably carcinogenic to humans (5). Depending on exposure and duration, lead compounds have toxic effects on human health which cause enzyme inhibition resulting in significant pathological conditions or death (6). Peripheral neuropathy and hypertension are the major concerns in adults including the immune, skeletal, nerve, and adrenal endocrine systems, and the respiratory tract (7). Even at low levels of exposure, haeme synthesis and other biochemical mechanisms can be damaged and neurobehavioural and psychological activities are weakened (8-10).

ABSTRACT

The objective of this study was to develop and validate a sensitive ICP-MS method with no gas mode for the determination of Pb levels in autopsy liver samples collected from patients in Diyarbakır, Turkey. The microwave-assisted acid digestion procedure was optimized and applied to accurately weighed dry liver samples. The method showed linearity in the range of 0-100 µg/kg with a detection and quantification limit of 0.72 µg/kg and 2.18 µg/kg, respectively. The calibration graph was characterized with excellent correlation coefficients ($r=0.9997$) for Pb determination. Certified reference material was used to validate the method in terms of accuracy, precision, and recovery. Good agreement was obtained between the certified values and the measured Pb concentrations. Percent recovery and precision for Pb was calculated at 99.5% and 4.29%, respectively. In the autopsy liver samples, the Pb levels ranged from 106.83 µg/kg to 1932.08 µg/kg and the mean Pb levels were found at 375.24 ± 325.46 µg/kg. As a result of the statistical analysis, the occupation, smoking habit, settlement, gender, and age appeared to have an effect on the Pb levels in the autopsy liver samples. The procedure described is relatively simple, precise, and applicable for routine toxicological Pb determination in human tissue.

Inductively coupled plasma mass spectrometry (ICP-MS) is one of the most sensitive analytical techniques

and provides the simultaneous determination of many heavy metals at ultra-trace levels in the different sample matrices (11). The first ICP-MS instrument was commercially marketed in 1983 and consists of six basic parts: sample input, plasma, sampling interface, ion directors, quadrupole (mass separation part) and detector part (12).

The main goal of the present study was to develop and validate a sensitive ICP-MS method with no gas mode for the determination of Pb levels in autopsy liver samples collected from patients in Diyarbakır, Turkey. In addition, it was also investigated whether occupation, smoking habit, settlement, gender and age has an effect on the Pb levels in autopsy liver samples.

EXPERIMENTAL

Instrumentation

The measurements were performed using a Model 7700x ICP-MS (Agilent Corporation, USA). The operating parameters of the ICP-MS system are listed in Table I.

Standard Solutions and Reagents

Multi-element calibration standard 2A solutions containing 100 mg/L lead and an ICP-MS internal standard mixture containing ^{209}Bi at the concentration of 1000 mg/L were purchased from Agilent Corporation, USA. Nitric acid (HNO_3 , 65% v/v) was obtained from Merck (Darmstadt, Germany). Ultrapure water (Merck Millipore Direct-Q8, Germany) with a resistivity of $18\text{M}\Omega\cdot\text{cm}$ was used to prepare the solutions for the experimental

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TABLE I
Operating Parameters for ICP-MS System

ICP-MS Instrumental Operating Parameters				
Ar Gas Pressure	540 kPa			
Forward power	1300 W			
Reflected power	7 W			
Cooling water flow rate (RF/WC/IF)	2.0 L/min			
Interface/Backing pressure (IF/BK)	252 Pa			
Analyzer Pressure in no gas mode	1.32 x 10 ⁻⁴ Pa			
RF Power	1550 W			
Sampling Depth	8.0 mm			
Omega Lens	7.3 V			
S/C Temp	2 °C			
Scanning mode	Peak hopping			
Isotopes	²⁰⁶ Pb, ²⁰⁷ Pb, ²⁰⁸ Pb			
Internal Standard	²⁰⁹ Bi			
Nebulizer	Meinhard™			
Spray chamber	Cyclonic			
Sampler and skimmer cones	Copper			
Shield torch	Platinum			
Autosampler	ASX-500 Series			
Bottle 1	Fresh double deionized water			
Bottle 2	Fresh 1% (v/v) HNO ₃			
Bottle 3	Tuning solution (1 µg/L ⁷ Li, ⁸⁹ Y, ²⁰⁵ Tl, and ¹⁴⁰ Ce in 2% HNO ₃)			
Water of chiller temperature	14 °C			
Low Matris Mode	< 0.1%			
Nebulizer Peristaltic Pump Operation				
	Time (sec) Speed (rps)			
Pre-Run	Sample Uptake 60 0.3			
	Stabilizing 25 0.1			
Post-Run	Probe Rinse 25 0.3			
	Rinse 25 0.1			
Ar gas flow rates (L/min)				
Plasma	16			
Auxiliary	1.0			
Nebulizer	1.0			
Sensitivity Values				
Mass	Count (cps)	RSD%	Background (cps)	W-10% (amu)
7	4566	3.812	3.7	0.741
89	17610	3.257	4.3	0.778
205	9098	4.153	20.2	0.834

process. Argon gas with a purity of 99.999% was bought from a local supplier (Linde Gaz, Diyarbakır, Turkey). The certified reference material (CRM) used for validation of the method was ERM®-BB 18 Pig Kidney (Environmental Resource Management, Brussels, Belgium).

Sample Collection

This study involved 100 mortal cases ranging from 18–90 years old. The liver samples were collected from the Ministry of Justice Diyarbakır Group Chairmanship of Council of Forensic Medicine in Diyarbakır, Turkey. The autopsy

liver tissue samples were taken within a maximum of 24 hours after the death certificate was issued. The samples were put into 50-mL polypropylene tubes and preserved in the deep-freezer at -25 °C until pretreatment before analysis. This study was ethically approved by the Dicle University Medical Faculty Ethics Committee for Noninterventional Studies (Decision No.: 04/25.12.2013). This work was also approved by the Turkish Ministry of Justice Council of Forensic Medicine (Reference No.: 03.09.2013 and B.03.1.ATK.0.01.00.08/529, respectively) in terms of sample collection from the autopsy cases.

Sample Preparation and Procedure

The autopsy liver tissues were placed on the vitreous platform and kept in a drying oven at 75 °C for 24 hours. After this step, the dried samples were accurately weighed, and the mean weight of the samples was 0.3584 ± 0.1206 g. Next, the samples were individually placed into temperature-resistant Teflon® tubes, pretreated by addition of 10 mL of 65% (v/v) nitric acid, sealed and placed into the microwave system (CEM Mars Xpress, Matthews, NC, USA) for digestion. The microwave temperature program is listed in Table II. The digested solutions were then diluted with ultra-pure water in 15-mL polypropylene tubes until the total volume of the samples reached 14 mL. The acid-digested autopsy liver samples were stored

TABLE II
Temperature Program for Microwave Digestion

Max. Power	600 W
Power	100%
Ramp Time	10 min
Pressure	350 psi
Temperature	200 °C
Hold Time	10 min

separately in the refrigerator at 4 °C until the day of analysis. In order to prepare calibration standards at the concentrations of 1.0, 5.0, 10.0, 25.0, 50.0, and 100.0 µg/L, a 100-µg/mL lead stock solution was diluted in 4% (v/v) HNO₃. An internal standard mixture containing ²⁰⁹Bi at the concentration of 1000 mg/L (Agilent, USA) was manually diluted with the use of nitric acid 4% (v/v) until the final concentration reached 100 µg/L. Then, the final solution of the internal standard was automatically pumped to all solutions. All glassware was kept in 10.0% (v/v) nitric acid for at least one night before the analysis.

Optimization

In order to achieve the best performance for this ICP-MS analysis, some important parameters were adjusted such as selection of concentration range in accordance with the elemental concentration in real liver samples and establishing linearity. The samples were injected (60 s, 0.3 rps) using a Meinhard® nebulizer with a chilled spray chamber. No flow injection valve was utilized, and the autosampler was directly set up to stay in the sampling position for the defined time. The argon plasma conditions were adjusted as forward power at 1300 W and reflected power of 7 W. The nebulizer gas flow rate, auxiliary and plasma gas flow rates were selected as 1.0, 1.0, and 16.0 L/min, respectively. Interface cones of nickel were used. The instrument was run in peak jumping mode while ²⁰⁶Pb, ²⁰⁷Pb, ²⁰⁸Pb, and ²⁰⁹Bi were scanned at three points per peak. The sum of the three lead isotopes was used for the calculations. For lead analysis in the autopsy liver samples, the dwell time was 0.07 s/point for lead and 0.02 s/point for Bi, and the acquisition time was 0.30 s/mass for lead and 0.15 s/mass for Bi. The method showed linearity in the range of 0-100 µg/L for lead

(Figure 1). The correlation coefficient (r) and the equation of the calibration curve for lead were respectively found to be r=0.9997 and Abs=0.1264*C+0.0339, where Abs stands for integrated absorbance and C is the lead concentration in µg/L.

Method Validation

To validate the method in terms of accuracy, precision, and recovery, the CRM ERM®-BB 18, Pig Kidney was analyzed 10 times with triplicate measurements. The precision of the methods is expressed as the relative standard deviation (RSD) of 10 independent analyses

of the CRM. The results were compared with the certified values for accuracy, precision, and recovery of the method. Good agreement was obtained between the certified values and the measured Pb concentrations. The percent recovery and precision for Pb were calculated as 99.5% and 4.29%, respectively. The analytical results of the CRM are listed in Table III.

Limit of Detection, Quantification, and Linearity

According to the ICH guidelines (13-15), the limit of detection (LOD) and lowest limit of quantification (LOQ) were determined

Fig 1. Calibration graph of Pb, performed by ICP-MS.

TABLE III
Analysis of Certified Reference Material

CRM	Number of Analyses (n)	Certified Value (µg/kg)	Measured Value (µg/kg)	Recovery (%)	RSD (%)
ERM-BB186 Pig Kidney	10	0.040±0.005	0.0389±0.0017	99.5	4.29

based on the standard deviation of the response and the slope of the calibration curve ($LOD=3.3\sigma/S$, $LOQ=10\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve). The LOD and LOQ for lead was equal to 0.72 $\mu\text{g/kg}$ and 2.18 $\mu\text{g/kg}$, respectively. The limit of linearity (LOL), which is the concentration at which the calibration curve departs from linearity, was 100 $\mu\text{g/L}$. The dynamic range refers to the concentration intervals from LOQ to LOL, and was found to be between 2.78 $\mu\text{g/kg}$ to 100.0 $\mu\text{g/kg}$ for lead. The schematic diagram of the dynamic range study is shown in Figure 2.

Statistical Analysis

The findings were statistically evaluated based on occupation (risky and unrisky), locality (rural and urban), smoking habit, age, and gender. The statistical analyses were performed utilizing the Statistical Package for Social Sciences (SPSS) version 16.0 software for Windows®. All results were expressed as the mean $\pm$ standard deviation (SD) of the mean, and the normality of data distribution was assessed with the Kolmogorov-

Smirnov test. The differences of the continuous variables between the two groups were evaluated by the Student's t -test and the correlations between the parameters were described using the Pearson's test. The statistical significance was considered as $p<0.05$.

RESULTS AND DISCUSSION

After exposure, lead accumulates in the soft tissue. According to autopsy studies, the liver appears to be the greatest depository of lead (33%), followed by the kidney cortex and medulla, pancreas, ovary, spleen, prostate, adrenal gland, brain, fat, testis, heart, and skeletal muscles (16, 17). Biological observation for human exposure to Pb indicates an individual's recent body burden as an occasion of present and/or previous exposure. Therefore, the relevant choice of biomarkers and determination of Pb exposure are highly important in terms of health care management, public health decision-making, and primary prevention activities (18). The World Health Organization (WHO) recommended the permissible weekly intake of Pb as 25.0 $\mu\text{g/kg}$ (body weight) (19). Thus,

for an adult at 70 kg, the allowable intake of Pb is 250 $\mu\text{g/day}$ (20).

In this study, the Pb levels of 100 autopsy liver samples were measured by ICP-MS and found to be between 106.83 $\mu\text{g/kg}$ and 1932.10 $\mu\text{g/kg}$. Thus, the mean Pb levels were 375.24 ± 325.46 $\mu\text{g/kg}$. As a result of the statistical analysis (see Table IV), occupation, smoking habit, living locality, gender, and age appeared to have an effect on the Pb levels in the autopsy liver samples. The mean liver lead content of the risky occupation group (523.29 ± 406.57 $\mu\text{g/kg}$) was statistically higher than the unrisky occupation group (280.59 ± 216.16 $\mu\text{g/kg}$) ($p=0.001$; $p<0.001$). The higher risk to lead exposure included drivers, cleaners, miners, and industrial workers. The effect of living in rural or urban areas on liver Pb levels was also investigated. It was found that the mean liver Pb concentration of individuals who had lived in urban areas (454.92 ± 374.14 $\mu\text{g/kg}$) was significantly higher than for rural areas (301.69 ± 255.19 $\mu\text{g/kg}$) ($p=0.018$; $p<0.05$). In an urban area, industrial pollution and occupational exposure risks are comparatively higher than for rural areas and is thus reflected in the higher liver lead levels of individuals who had lived in urban rather than rural areas.

Since lead is one of the many chemicals found in cigarettes, smokers are easily exposed to lead. According to another finding of this study, the Pb levels in smokers (455.95 ± 366.92 $\mu\text{g/kg}$) were significantly higher than for nonsmokers (254.18 ± 200.39 $\mu\text{g/kg}$, $p=0.002$; $p<0.05$). In addition, significantly higher values of Pb were found in males (413.49 ± 357.39 $\mu\text{g/kg}$) than in females (247.22 ± 114.72 $\mu\text{g/kg}$, $p=0.031$; $p<0.05$). Furthermore, a positive correlation was observed between liver Pb levels and age. Hence, liver Pb levels increased significantly with age as well ($r = +0.204$ and $p = 0.042$; $p<0.05$).

Fig 2. Schematic of dynamic range study. ICP-MS method for lead analysis in autopsy liver samples provided LOD, LOQ, and LOL equal to 0.72 $\mu\text{g/kg}$, 2.18 $\mu\text{g/kg}$ and 100.0 $\mu\text{g/kg}$, respectively.

Since all findings obtained from this study were carried out in Diyarbakır, Turkey, they will be a good reference for future studies in the assessment of lead levels in autopsy liver samples in other locations and nations.

CONCLUSION

ICP-MS has proven to be the optimal methodology for pursuing the lowest achievable detection limits and highest level of productivity in the Pb analysis of autopsy liver samples. In this work, the LOD, LOQ, and LOL values were equal to 0.72 µg/kg, 2.18 µg/kg, and 100.0 µg/kg, respectively. The percent recovery and precision for Pb was found at 99.5% and 4.29%, respectively. The ICP-MS technique is almost free from interferences, relatively simple, precise, and applicable for routine postmortem toxicological Pb analysis.

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TABLE IV
Statistical Analysis (µg/kg)

Groups	Minimum	Maximum	Mean±S.D.	p Value
Occupation				
Unrisky (n=61)	106.83	457.20	280.59±216.16	0.001**
Risky (n=39)	278.20	1932.08	523.29±406.57	
Settlement				
Rural (n=52)	106.83	610.20	301.69±255.19	0.018*
Urban (n=48)	235.80	1932.08	454.92±374.14	
Smoking Habit				
Smoking (n=60)	275.99	1932.08	455.95±366.92	0.002**
Non-Smoking (n=40)	106.83	455.45	254.18±200.39	
Gender				
Female (n=23)	106.83	608.10	247.22±114.72	0.031*
Male (n=77)	111.09	1932.08	413.49±357.39	

* p value < 0.05

** p value ≤ 0.01