

골중납 측정의 시료 전처리 (산분해법과 마이크로웨이브 분해법)와 측정 방법 (유도결합 플라즈마 질량분석법과 흑연로 원자 흡수 분광 광도법)의 비교

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Comparisons of sample preparation (acid digestion and microwave digestion) and measurement (inductively coupled plasma mass spectrometry and graphite furnace atomic absorption spectrometry) in the determination of bone lead.

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요 약 : 본 연구는 인체의 골중 납 농도를 측정하는데 필요한 시료 전처리법과 기기 분석법을 평가하기 위하여 수행되었다. 절단한 인간 사체 다리의 뼈를 상온에서의 산 분해법과 마이크로웨이브 분해법 (microwave oven method)을 이용하여 납을 추출하였으며, 유도결합 플라즈마 질량분석법 (ICP-MS)과 흑연로 원자 흡수 분광 광도법 (GF-AAS)으로 납 농도를 측정하였다. 표준시료를 이용하여 회수율을 조사한 결과, 산분해 후 ICP-MS로 측정한 경우 높은 회수율을 보였으나, 산분해 후 GF-AAS로 측정한 것은 표준 농도보다 낮은 값을 보였으며, 마이크로웨이브 분해한 경우는 두 측정기기 모두 표준 농도보다 높은 값을 보였다. 뼈 시료의 경우, 산분해 후 ICP-MS로 측정한 납 농도는 GF-AAS의 값과 높은 상관성을 보였으나 (상관계수 = 0.983), GF-AAS가 ICP-MS보다 계통적으로 높은 값을 측정하였다. 마이크로웨이브 분해 역시 두 분석방법이 높은 상관성을 보였으나 (상관계수 = 0.950), 대체로 산분해에 의한 값보다 높은 농도를 보였다. 결론적으로, 상온에서 질산으로 분해 후 ICP-MS를 이용하여 측정한 것이 골중 납 농도를 결정하는데 편이성과 정확성 면에서 가장 효율적인 방법으로 보인다.

Abstract : This study was conducted to evaluate two sample digestion procedures and instrumental determination parameters for analysis of lead in bone. Amputated human legs were treated by acid digestion or microwave dissolution prior to spectrometric analysis. Inductively coupled plasma mass

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spectrometry (ICP-MS) and graphite furnace atomic absorption spectrometry (GF-AAS) were used for determining bone lead levels. Recovery efficiencies using standard reference material from acid digestion measured by ICP-MS were in good agreement with those of the certified value, but in cases of acid digestion by GF-AAS and microwave digestion by both two methods, recovery underestimated and overestimated, respectively. For the bone samples, the lead concentrations obtained by ICP-MS after acid digestion were in good agreement with those by GF-AAS (correlation coefficient = 0.983), but GF-AAS gave systematically higher values than ICP-MS. While a good agreement between two analytical methods after microwave digestion was also obtained (correlation coefficient = 0.950), bone lead concentrations from microwave were relatively higher than those from acid digestion. In conclusion, the use of the simple nitric acid digestion procedure at an ambient temperature coupled to ICP-MS seems to be efficient for the determination of lead in bone in consideration for both the convenience and validity.

Key words : bone lead, acid digestion, microwave oven, SRM, ICP-MS, GF-AAS

1. INTRODUCTION

Recent studies have shown that lead, even at relatively low levels of exposure, has the potential to harm not only the young and the occupationally exposed, but also older people.¹ The skeleton is the primary site of storage for 90-95% of lead in the adult human body. Because of its long half-life, bone lead reflects cumulative exposure to lead.² Therefore bone lead is an ideal marker to assess the long-term exposure of an environmentally exposed population.

Several methods, such as inductively coupled plasma mass spectrometry (ICP-MS), graphite furnace atomic absorption spectrometry (GF-AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and instrumental neutron activation analysis (INAA), are available for the determination of lead in bone. GF-AAS is most commonly used at present for lead determination, because not only it is more available but also the procedure is relatively simple and fast and its detection limit is low enough for analysis of most environmental and biological materials.³ The lead concentration in bone was determined in a piece of pelvic bone taken in vivo by a biopsy using GF-AAS. GF-AAS has excellent sensitivity for lead and would be an ideal choice for the analysis of

small samples of bone. However, the determination of extremely small quantities of lead in bone may require the use of more sensitive methods such as ICP-MS.⁴ Therefore, ICP-MS has been known that it is very effective method to detect the concentration of bone lead in low exposed population. Also, if many samples need to be analyzed for a number of elements, as is often encountered in environmental health studies, ICP-MS is recommended.

In addition, when lead was determined by spectrometric techniques, various digestion procedures were applied.⁵ Bone samples were dry ashed and the ash was dissolved in nitric acid^{6,7}, or nitric acid digestion was applied at normal pressure⁸⁻¹⁰ or in a microwave digestion system.¹¹ Microwave acid digestion methods have been used recently because of simplicity and effectiveness by high temperature and high pressure for biological samples.¹¹ The advantages of a closed microwave digestion system are minimal reagent consumption, complete digestion, rapid dissolution, sample integrity which allows volatile element determination, lower reagent blank and potential automation.¹² For this reason, recent studies have adopted microwave digestion method.^{5,11,13}

The purpose of this study was to develop a reliable and accurate analytical method of digestion for the

determination of lead in bone using concentrated nitric acid and microwave by ICP-MS and GF-AAS. Accuracy of the method here has been demonstrated by comparison of the results obtained with those of the National Institute of Standard and Technology (NIST) Standard Reference Material (SRM) 1400 bone ash and 1486 bone meal.

2. MATERIALS AND METHODS

2.1. Instrumentation

A microwave oven (Mars 5, CEM, USA) was used for the digestion of bone samples. Samples were analyzed by both inductively coupled plasma-mass spectrometer (ICP-MS, Ultramass, Varian, Australia) and graphite furnace (GTA-100, Varian, Australia)-atomic absorption spectrometer (GF-AAS, Spectraa 880, Varian, Australia).

2.2. Reagents

Redistilled 70% nitric acid (>99.999% purity, Aldrich, USA) and type III water (purified by milli-Q, Millipore, USA) were used for cleaning of laboratory ware, digestion and preparation of standard solutions. All other chemicals were of analytical-reagent grade.

Fresh working standard solutions were prepared by dilution of ICP-MS multi standard stock solution (10 ug/ml lead in 5% HNO₃, QCP-QCS-3, Inorganic ventures, Inc, USA) with distilled water.

2.3. Sample preparation

The tibia bone samples, amputated around the knee were obtained from the Department of Anatomy, College of Medicine, Seoul National University. They had some overlying tissue and had been preserved with formalin to avoid decomposition. Bone samples were scraped to remove adhering tissue, periosteum, and marrow, rinsed with acetone to defat, and dried at room temperature.

For the room temperature acid digestion method, bone samples were weighed (about 2-5 g) in quartz crucibles and then 15 mL of concentrated HNO₃ acid was added to each sample, and samples were covered and digested at

room temperature for 3 days. The digested samples were evaporated to dryness on a hot plate before ashing in a muffle furnace at 500 °C for 10 hours. After complete ashing in a muffle furnace, ashed samples (0.300±0.002 g) were weighed and dissolved in 50 mL of 10% HNO₃ acid for lead analysis.

For the microwave digestion, 1-2 g of dry bone samples was exactly weighed into a 100 mL PTFE vessel. A 7.1 mL of concentrated HNO₃ was added and the sample digested in a closed microwave system at a power of 1200 W following the ramp to pressure program. In the first 25 min, the pressure was raised to 170 psi (or 210 °C) and was held at 170 psi (or 210 °C) for the next 10 min. After microwave oven digestion, samples were diluted to 50 mL of 10% HNO₃ solution for analysis. All glassware and crucibles to contact the samples and standards were soaked in diluted HNO₃ for enough times (over 10 hours) during the cleaning process, and all the sample handling was performed in a clean hood.

2.4. Determination of lead by ICP-MS and GF-AAS

Samples were analyzed using ICP-MS and GF-AAS. Three replicate measurements of Pb concentration in each bone sample were made. The average Pb concentration and standard deviation of the three values was used in subsequent calculations. Typical operating parameters are listed in *Table 1* and *Table 2*, respectively.

Table 1. ICP-MS conditions for the analysis of bone lead concentration

Spectrometer	Ultramass (Varian, Australia)
Analysis mode	Scan mode: Peak hopping, Scans/Replicate: 100, Replicates/Sample: 3 Power: 1.2 kW, Flow: 15 L/min,
Plasma	Auxiliary flow: 1.3 L/min, Nebulizer flow: 0.89 L/min, Sampling depth: 6.0 mm
Resolution	0.80 amu
Optimization	At masses ⁹ Be, ¹¹⁵ In, ²⁰⁸ Pb, ¹³³ Cs, ¹³⁸ Ba
Instrument detection limit	1 ppb
Internal standard	100 ppb ²⁰⁹ Bi

Table 2. GF-AAS conditions for the analysis of bone lead concentration

Spectrometer	AA-100 (Varian, Australia)
Wavelength	283.3 nm
Slit	0.7 nm
Injection volume	20 $\mu\ell$
Background	On
	Drying (Temp. 100/130 $^{\circ}\text{C}$; Ramp 20/10 s; Hold 10/10 s)
	Ashing (Temp. 500 $^{\circ}\text{C}$; Ramp 10 s; Hold 10 s)
Furnace program	Atomization (Temp. 1100 $^{\circ}\text{C}$; Ramp 0 s; Hold 5 s)
	Cleaning (Temp. 2650 $^{\circ}\text{C}$; Ramp 1 s; Hold 2 s)
Furnace tube	Pyrolytic

The overall accuracy of the ICP-MS and GF-AAS measurements by means of digestion methods was evaluated by analysis of bone meal standard reference material (NIST SRM 1486), which underwent exactly the same sample preparation as the bone samples. We also analyzed bone ash standard reference material (NIST SRM 1400), which assured the accuracy of the ICP-MS and GF-AAS without digestion step. About 0.3 g of SRM 1400 were exactly weighed and diluted to 50 ml with 10% HNO_3 recovery of lead concentration of these samples was represented.

The laboratory in which ICP-MS and GF-AAS measurements have been performed is involved in Environmental Lead Proficiency Analytical Testing (ELPAT) program provided by US National Institute for Occupational and Safety Health American Industrial Hygiene Association (NIOSH-AIHA).¹⁴

3. RESULTS AND DISCUSSION

3.1. Recovery efficiencies of lead in NIST SRM 1400 Bone Ash reference material and NIST SRM 1486 Bone Meal reference material

Recovery efficiencies (%) of lead in SRM 1400 are shown

in Table 3. Analytical values were in good agreement with the certified value regardless of analytical instruments. There was no statistical significance ($p>0.05$) between recovery of ICP-MS (101.8%) and that of GF-AAS (100.7%).

Table 3. Lead concentrations obtained for the NIST SRM 1400 Bone Ash¹ reference materials with ICP-MS and GF-AAS

ICP-MS		GF-AAS	
Found \pm SD ($\mu\text{g/g}$)	Recovery (%)	Found \pm SD ($\mu\text{g/g}$)	Recovery (%)
9.278 \pm 0.121	102.29	9.245 \pm 0.083	101.93
9.150 \pm 0.261	100.89	8.956 \pm 0.017	98.74
9.279 \pm 0.191	102.30	9.192 \pm 0.028	101.35

¹Certified value: 9.07 \pm 0.12 $\mu\text{g/g}$

Recovery efficiencies (%) of lead in SRM 1486 according to both digestion and measurement methods are shown in Table 4. Recovery from acid digestion measured by ICP-MS was 97.3 (91.0 ~ 100.8)%, found to be in good agreement with those of the certified value. In the case of GF-AAS, however, recovery from acid digestion was relatively low, 92.2 (83.4 ~ 96.9)%.

Results of analysis of the standard reference material from acid digestion indicate acceptable accuracy and recovery for lead in bone.

On the other hand, the microwave digestion method at both ICP-MS and GF-AAS overestimated lead concentration. Recovery by both analytical methods was more than 120% but there was no statistical significance between ICP-MS and GF-AAS (126.86% vs. 126.85%, $p>0.05$).

The possible reason of high recovery of microwave digestion samples was sample matrix. In acid digestion method, samples were first digested at room temperature for 3 days and more completely ashed at high temperature (500 $^{\circ}\text{C}$) for 10 hours. In microwave oven, bone samples were treated within a closed vessel for a relatively short duration. We use Bi 209 as an internal standard in ICP-MS analysis. This means that lead concentration of the sample was corrected with Bi response ratio in comparison with Bi response of blank solution. Average

Table 4. Lead concentrations obtained for the NIST SRM 1486 Bone Meal¹ reference materials with ICP-MS and GF-AAS after acid digestion and microwave digestion

Digestion	ICP-MS		GF-AAS	
	Found \pm SD ($\mu\text{g/g}$)	Recovery (%)	Found \pm SD ($\mu\text{g/g}$)	Recovery (%)
Acid digestion	1.124 \pm 0.051	90.91	1.113 \pm 0.043	83.35
	1.336 \pm 0.038	100.08	1.288 \pm 0.005	96.48
	1.345 \pm 0.025	100.78	1.294 \pm 0.003	96.89
Microwave digestion	1.596 \pm 0.101	119.52	1.657 \pm 0.003	124.09
	1.792 \pm 0.069	134.20	1.730 \pm 0.017	129.60

¹Certified value: 9.07 \pm 0.12 $\mu\text{g/g}$

Bi ratio of acid digestion SRM 1486 samples was 0.44 whereas microwave digestion sample was 0.31, which means low response when microwave digestion samples were introduced into the ICP-MS because of complex matrix. Therefore, lead levels of microwave digestion samples, corrected by low internal standard response ratio, were measured relatively high. Bone was composite material of organics and inorganic. We assume much amount of bone matrix were removed with acid digestion technique because of high temperature (500 °C) and long duration (10 hours). At this ppb analytical level, another possible reason was unobserved contamination.

3.2. Determination of lead in bone samples by ICP-MS and GF-AAS

We have determined the levels of lead in 10 tibia bone samples (Table 5). Lead concentrations after acid digestion measured by ICP-MS and GF-AAS ranged from 3.41 to 12.74 $\mu\text{g Pb/g}$ bone ash and 4.02 to 13.40 $\mu\text{g Pb/g}$ bone ash, respectively. The lead concentrations obtained by ICP-MS were in good agreement with those by GF-AAS (correlation coefficient = 0.983), but GF-AAS gave systematically higher values than ICP-MS (Fig.1). Paired t-test showed that there was a significant difference between ICP-MS and GF-AAS measurements ($p = 0.004$).

Table 5. Analysis of lead in bone after acid digestion and microwave digestion by ICP-MS and GF-AAS¹

Sample pair	Acid digestion ²		Microwave digestion	
	ICP-MS	GF-AAS	ICP-MS	GF-AAS
1	3.41 \pm 0.18	4.02 \pm 0.01	3.74 \pm 0.02	3.94 \pm 0.15
2	3.73 \pm 0.08	4.07 \pm 0.05	3.95 \pm 0.06	3.67 \pm 0.01
3	4.18 \pm 0.08	4.52 \pm 0.01	12.41 \pm 0.33	10.45 \pm 0.84
4	4.35 \pm 0.10	5.15 \pm 0.02	5.45 \pm 0.03	5.29 \pm 0.16
5	4.90 \pm 0.15	5.20 \pm 0.24	5.68 \pm 0.25	5.59 \pm 0.12
6	5.39 \pm 0.16	7.58 \pm 0.01	7.02 \pm 0.16	6.10 \pm 0.29
7	6.32 \pm 0.13	6.67 \pm 0.01	14.00 \pm 0.44	12.68 \pm 0.37
8	7.43 \pm 0.20	8.08 \pm 0.01	4.66 \pm 0.16	4.53 \pm 0.03
9	9.55 \pm 0.20	10.10 \pm 0.05	9.67 \pm 0.11	8.87 \pm 0.36
10	12.74 \pm 0.33	13.40 \pm 0.03	23.13 \pm 0.69	36.67 \pm 0.18

¹Results are expressed on a dry weight basis, as the mean of three parallel determinations \pm standard deviation of measurement ($\mu\text{g Pb/g}$ bone ash).

²There is a significant difference between ICP-MS and GF-AAS measurements for acid digestion method ($p = 0.004$, paired t-test).

Bone lead levels after microwave digestion ranged from 3.74 to 23.13 $\mu\text{g Pb/g}$ bone ash by ICP-MS and 3.67 to 36.67 $\mu\text{g Pb/g}$ bone ash by GF-AAS, that is, in general, as determined in NIST SRM 1486, higher than those by acid digestion (Table 5). A good agreement between the two analytical methods was also obtained (correlation coefficient = 0.950), but contrary to acid digestion, no significant statistical difference was found between the two methods ($p = 0.59$, by paired t-test).

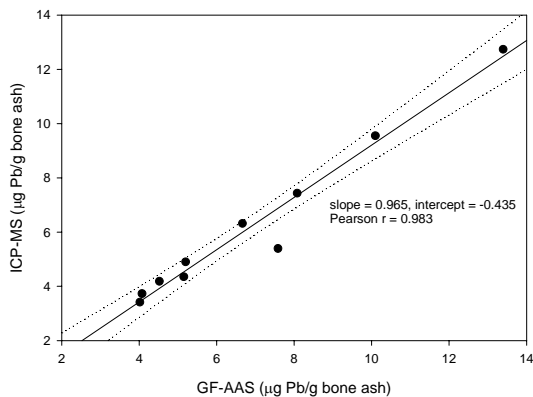


Fig. 1. Correlation of the results obtained for the determination of lead in bone between ICP-MS and GF-AAS for acid digestion method.

Zong et al. (1996)¹⁵ compared wet digest and microwave digest for lead concentration in bone and found that the data were highly consistent, but wet digest was a time consuming process compared to microwave digest and there is a greater potential for contamination from nitric acid in wet digest. Siitonen and Thompson (1998)¹⁶ compared the results of lead and calcium in calcium supplements after microwave dissolution or dry-ash digestion, and reported that data from microwave dissolution procedure gave slightly better statistics for calcium, but data from dry-ash digestion gave slightly better lead statistics. In this study, data from microwave digestion showed higher concentrations than real values with the SRM 1486 and bone samples. So, we found that traditional acid digestion method gave more accurate results.

4. CONCLUSION

The use of the "simple" nitric acid digestion procedure at an ambient temperature coupled to ICP-MS seems to be efficient for the determination of lead in bone. Although several previous studies emphasized the advantages of microwave digestion, in case of complex biological samples such as bone, acid digestion may be more reliable method than microwave digestion. Though there was no statistical difference for NIST SRM 1400 bone ash samples between ICP-MS and GF-AAS, ICP-MS results tend to be lower than those of GF-AAS for real human tibia bone samples.

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