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Comparative Evaluation of GFAAS and ICP-MS for Analyses of Cadmium in Blood

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Abstract: Cadmium in blood (Cd-B) is an important indicator, next to Cd in urine, in biological monitoring of exposure to Cd. The present study was initiated to examine compatibility in results of analysis for Cd-B between graphite furnace atomic absorption spectrophotometry (GFAAS) and inductively-coupled plasma mass-spectrometry (ICP-MS). For this purpose, 1,159 blood samples were collected from adult women (with no occupational exposure to Cd) in eight prefectures nation-widely in Japan. The samples were analyzed by the two methods; geometric mean (the maximum) concentrations were 1.22 (6.90) μg/l by ICP-MS, and 1.47 (7.40) μg/l by GFAAS. Statistical analyses showed that there was a close correlation between the results by the two methods. The regression line (with ICP-MS and GFAAS results as an independent variable and a dependent variable, respectively) had a slope close to one and an intercept next to zero to suggest that ICP-MS gave values compatible with that of GFAAS. Further analysis with the ratio of Cd-B by GFAAS over that by ICP-MS revealed that the two results were close to each other, and that the agreement was even closer when Cd-B was >2 μg/l. Thus, the two methods can be employed inter-convertibly when Cd-B is relatively high, e.g. >2 μg/l. Care may need to be practiced, however, for possible 'between methods' difference when Cd-B is low, e.g., ≤2 μg/l.

Key words: Blood, Cadmium, GFAAS, ICP-MS, Population with no occupational exposure

Introduction

Cadmium (Cd) is a typical toxic pollutant metal ubiquitous in the general environment[1)], and monitoring for Cd concentration in biological materials is one of the most common procedures to assess Cd exposure of working populations with occupational exposure to this metal as well as general populations with Cd exposures via general environment[2–4)].

With regard to the methods of analysis for biological exposure monitoring, graphite furnace atomic absorption spectrophotometry (GFAAS) has been enjoying high reputation as a reliable method for determination of Cd in biological materials such as blood and urine[4)]. In recent years, increasing attention has been paid to inductively-coupled plasma-mass spectrometry (ICP-MS) as a method for metal analysis with the instrument and material limits of determination substantially lower than that for GFAAS[5, 6)]. Nevertheless, studies on the compatibility between the two methods are few in literature; only two reports[7, 8)] are available with conflicting results.

It was the purpose of the present study to examine, with more than 1,000 samples, if cadmium in blood (Cd-B) measured by ICP-MS agrees with that by GFAAS. Efforts were further extended to find critical Cd-B levels below which discrepancy would be large enough to be a matter of concern.

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Subjects, Materials, Methods and Ethical Issues

Subjects and materials

Peripheral blood samples were collected from adult women (mostly non-smoking) in 2002 to 2008 in eight prefectures nation-widely in Japan.

Ethical issues

The study protocol was approved by the Ethics Committee, Kyoto Industrial Health Association, Kyoto, Japan, and each of the participating women provided her informed consent in writing.

Blood analyses for Cd

Analyses for Cd-B were conducted by two methods. For GFAAS analysis, 200 μl of blood was mixed with 100 μl of 10% Triton in water, 200 μl of 10% diammonium hydrogen phosphate in water and 200 μl of 0.1 N nitric acid. A 10 μl portion of the mixture was introduced to a GFAAS (Hitachi polarized Zeeman atomic absorption spectrophotometer, Z-5710, Hitachi High-technologies Corp., Tokyo, Japan) by use of an auto-sampler. The measurement was made at 288.8 nm under the operation conditions previously described in detail[9]. In case of ICP-MS analysis, an aliquot (100 μl) of each blood sample was digested in presence of 500 μl of 68% (about 14.8N) nitric acid in a closed vessel by the microwave method, and the digest was taken up in 5 ml of ultra-pure water. The solution was introduced to an ICP-MS system (Thermo Scientific ELEMENT2 High Performance High Resolution ICP-MS, Thermo Fisher Scientific Inc, Bremen, Germany) as previously detailed[6]. The standard addition method was employed for quantification in both analytical methods.

Data on Cd-B measured by ICP-MS were cited from Ikeda and others[6], whereas Cd-B analyses by GFAAS were conducted anew for the present study. In practice, complete data sets (i.e., Cd-B both by ICP-MS and by GFAAS) were available for 1,159 cases. The method limit of determination (LOD) for Cd-B analysis by ICP-MS was 0.1 μg/l[6], and that by GFAAS was 0.5 μg/l as previously described[8]. The quality of analysis for Cd both by ICP-MS and by GFAAS was approved by G-EQUAS (External Inter-comparison Programme 2002 and 2009).

Quality control standard material

The quality control standard material for trace elements in human whole blood consisted of two preparations of Level 1 (SERO201505; Cd-B $\fallingdotseq$ 0.7 μg/l) and Level 2 (SERO201605; Cd-B $\fallingdotseq$ 6 μg/l), and was supplied by SERO AS, Billingstad, Norway.

Statistical analyses

A normal distribution was considered for age and a log-normal distribution for Cd-B[1–3], so that the distributions of the former and the latter were expressed in terms of arithmetic means (AMs) ± arithmetic standard deviations (ASDs) and geometric means (GMs) [geometric standard deviations (GSDs)], respectively. In some instances, medians (MEDs), and the minimum (Min.) and the maximum values (Max.) were also shown. In case the analyte concentration was below LOD (in practice, such was observed only in 17 cases of Cd-B by GFAAS), a half the LOD was taken in the place. Parametric *t*-test was applied to detect possible difference between the pairs, before or after logarithmic conversion as necessary. Regression analysis and correlation matrix analysis were also employed.

Results

Analyses of quality control standard materials by ICP-MS and GFAAS

The pair of the standard materials (Level 1 and Level 2) was analyzed by ICP-MS and by GFAAS (Table 1). The standard values for quality control attached to the materials were given in terms of AM and the 95% confidence intervals (the 95%CI), one set each for ICP-MS and for GFAAS. The AM and ASD for the values measured in the present study were based on 10 deter-

Table 1. Comparison of experimental values with quality control standard values

Reference material	ICP-MS		GFAAS	
Parameter	Standard	Experimental	Standard	Experimental
Level 1				
AM	0.74	0.69	0.7	0.78
ASD		0.03		0.03
95%CI	0.68 to 0.80		0.4 to 1.0	
CV (%)	49.1	4.6	26.0	3.6
Recovery (%)		93		111
				105[a]
Level 2				
AM	6.0	6.04	5.1	6.92
ASD		0.57		0.22
95%CI	5.6 to 6.4		2.8 to 7.4	
CV (%)	4.0	9.5	27.3	3.2
Recovery (%)		101		136
				115[a]

Unless otherwise specified, the values in the table are in μg/l.
AM, ASD, CI and CV stand for arithmetic mean, arithmetic standard deviation, confidence interval and coefficient of variation (i.e., ASD/AM in %), respectively.
Recovery is defined as experimental value/certified value in %.
n=10 for experimental values by GFAAS and ICP-MS.
[a]Recovery in reference to the standard values for ICP-MS

minations each.

The AM experimental values for ICP-MS and GFAAS were all in the corresponding 95% confidence intervals. CV values (i.e., ASD/AM in %) were 4.6 (Level 1) and 9.5% (Level 2) for ICP-MS, and 3.6 (Level 1) and 3.2% (Level 2) for GFAAS, the former CVs being larger than the latter values. The recovery rate (i.e., the rate of experimental value over the standard value for quality control in AM) was 93 (Level 1) and 101% (Level 2) for ICP-MS, and 111 (Level 1) and 136% (Level 2) for GFAAS.

Basic parameters

The distributions of age and Cd-B as measured by ICP-MS and GFAAS are summarized in Table 2. The mean age of the participants was 43.7 yr scattering in a wide range of 20 to 74 yr. Age correlated weakly but significantly with Cd-B (r=0.184 for Cd-B by ICP-MS, and 0.215 for Cd-B by GFAAS; $p<0.01$ for both coefficients).

GM for Cd-B as measured by ICP-MS, 1.22 μg/l, was slightly smaller (by 17%) than that by GFAAS (1.47 μg/l). Comparison of the two values before and after logarithmic conversion by paired *t*-test showed that the difference was significant ($p<0.01$) irrespective of logarithmic conversion.

Table 2. Basic parameters

Parameter[a]	Age (yr)	Cd-B by		Ratio[b]
		ICP-MS (μg/l)	GFAAS (μg/l)	
GM	43.7[c]	1.22	1.47	1.2
GSD	10.2[c]	1.70	1.66	1.0
MED	44	1.2	1.5	1.3
Min.	20	0.1	<LOD[d]	NC[e]
Max.	74	6.9	7.4	1.1

In total, 1,159 cases were studied.
[a]AM, ASD, GM, GSD, MED, Min. and Max. stand for arithmetic mean, arithmetic standard deviation, geometric mean, geometric standard deviation, median, the minimum and the maximum, respectively.
[b]The ratio of Cd-B by GFAAS (μg/l) over Cd-B by ICP-MS (μg/l).
[c]AM and ASD in place of GM and GSD.
[d]LOD; 0.1 μg/l for ICP-MS and 0.5 μg/l for GFAAS.
[e]Not calculable.

Difference in Cd-B between ICP-MS and GFAAS as a function of Cd-B

Regression analyses taking Cd-B by ICP-MS as an independent variable and that by GFAAS as a dependent variable (the first line in Table 3) showed that there was a close correlation (r=0.964, $p<0.01$) between the pairs, and the slope was close to one (i.e., 1.059) whereas the intercept was next to zero. Similar analysis with the values after logarithmic conversion (the second line in Table 3) gave essentially the same results with one difference that the slope was slightly smaller than one (i.e., 0.895). Plotting of the paired values on the ordinary scales taking Cd-B by ICP-MS on the horizontal (X) axis and that by GFASS on the vertical (Y) axis showed that there was a close correlation between the pairs with a regression line close to Y=X but the range of scattering around the regression line appeared to be wider as a reverse function of Cd-B (Fig. 1). The trend of wider scattering at lower Cd-B was clearer when the

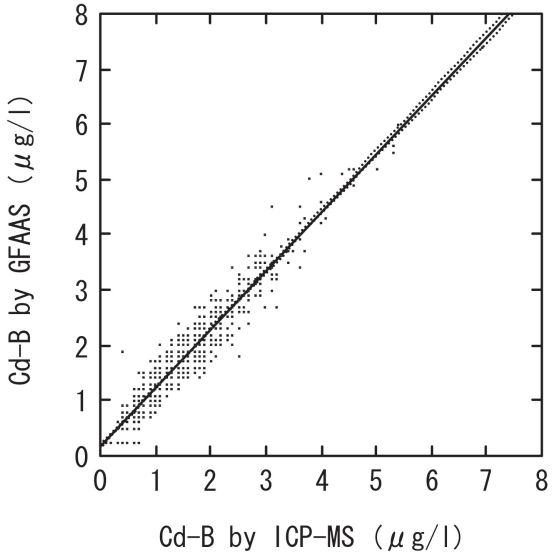

Fig. 1. Correlation of Cd-B values as measured by ICP-MS and by GFAAS.
Each dot represents one case (n=1,159).
The line in the middle is a calculated regression line (Y=0.174+1.059X), and two dotted curves on both sides show the 95% confidence interval.

Table 3. Parameters of regression analysis

Independent variable	Dependent variable	Intercept	(95%CI)	Slope	(95%CI)	r	*p* for r	Note
Cd-B by ICP-MS (μg/l)[a]	Cd-B by GFAAS (μg/l)	0.174	(0.147 to 0.201)	1.059	(1.042 to 1.076)	0.964	<0.01	Fig. 1
log [Cd-B by ICP-MS (μg/l)][a]	log [Cd-B by GFAAS (μg/l)]	0.089	(0.084 to 0.093)	0.895	(0.876 to 0.9141)	0.938	<0.01	Fig. 2
Cd-B by ICP-MS (μg/l)[a]	The ratio[c]	1.348	(1.321 to 1.376)	–0.090	(–0.107 to –0.073)	0.291	<0.01	Fig. 3
Cd-B by ICP-MS (μg/l)[b]	The ratio[c]	1.155	(1.015 to 1.295)	–0.012	(–0.049 to 0.025)	0.092	>0.10	

[a]n=1,159.
[b]Cd-B >3 μ/g (by ICP-MS) was selected (n=52).
[c]The ratio of [Cd-B by GFAAS (μg/l)] over [Cd-B by [ICP-MS (μg/l)].

values were logarithmically converted (Fig. 2). For further analysis, the ratio of Cd-B by GFAAS over Cd-B by ICP-MS was calculated for each case and plotted against Cd-B by ICP-MS. The plotting (Fig. 3) showed that the ratio scattered in a wider range when Cd-B was smaller (e.g., <1 μg/l) but tended to converge toward a horizontal line as a function of increasing Cd-B (e.g. ≥2 μg/l).

Figure 3 might be misleading because more cases were available in lower Cd-B ranges and the variation in the ratio could be greater simply due to a larger number of the cases. Thus, slopes of the regression lines and coefficients of variation were compared for Cd-B of different concentration ranges. Cases were classified in terms of a Cd-B range of 1.0 μg/l (starting from <LOD) and with an increase of 0.5 μg/l (i.e., <LOD to <1.0 μg/l, 0.5 to <1.5 μg/l, 1.0 to <2.0 μg/l and so on; Table 4). The slope for all cases in combination was negative (<0), i.e., –0.090, with the 95%CI of –0.107 to –0.073) but it was essentially zero when cases with Cd-B ≥3 μg/l (n=52) were selected (the bottom line in Table 3). In fact, the slope was already zero in the Cd-B range of 2 to <3 μg/l (the 95% range for the slope of –0.075 to 0.057, n=156; in the middle of Table 4). In contrast, the slope for Cd-B range of <2 μg/l was negative, i.e., –0.455 to –0.131, and the upper limit of 95% range was also less than zero (Table 4). Coefficients of variation converged to 10% in the higher Cd-B ranges.

After convergence to a horizontal line, the intercept for the fourth equation in Table 4 (with 52 cases in the Cd-B range of ≥3 μg/l) was 1.155 (the 95%CI; 1.015 to 1.295). The observation suggests that GFAAS would

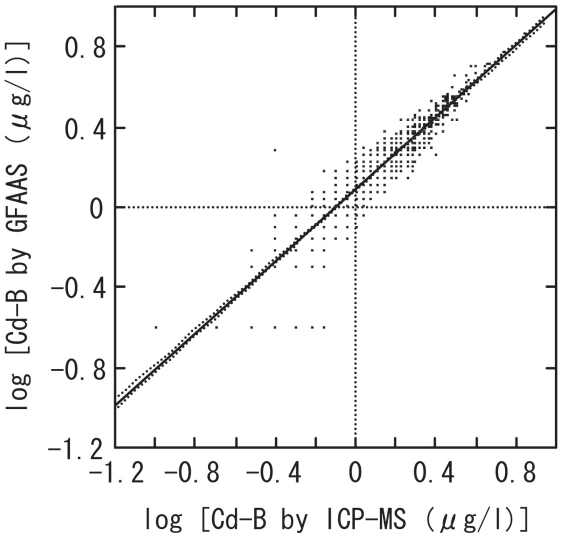

Fig. 2. Correlation of log Cd-B values as measured by ICP-MS and that by GFAAS.
The meaning of the line (Y=0.089+0.895X), two curves and dots are as in Fig. 1.

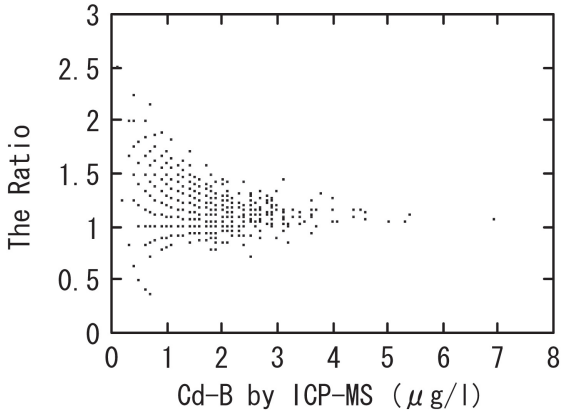

Fig. 3. Relationship of Cd-B (by ICP-MS) with the ratio of Cd-B by GFAAS over that by ICP-MS.
Note a wide dispersion when Cd-B is low (e.g., <2 μg/l) and a conversion to a zone of the ratio =1.0 to 1.2 when Cd-B is high (e.g., >4 μg/l).

Table 4. Changes in slope as a function of Cd-B

Cd-B[a]			The ratio[b]			Regression line			
Range	n	MED	AM	ASD	CV	Slope	(95%CI)	|r|	*p*
<LOD[c] to 1.0	357	0.7	1.32	0.35	26.1	–0.455	(–0.657 to –0.252)	0.228	<0.01
0.5 to 1.5	692	1.0	1.25	0.22	17.2	–0.211	(–0.270 to –0.152)	0.257	<0.01
1.0 to 2.0	594	1.3	1.19	0.16	13.6	–0.131	(–0.178 to –0.084)	0.219	<0.01
1.5 to 2.5	317	1.8	1.15	0.14	12.1	–0.085	(–0.139 to –0.030)	0.170	<0.01
2.0 to 3.0	156	2.4	1.13	0.12	10.7	–0.009	(–0.075 to 0.006)	0.022	>0.10
2.5 to 3.5	87	2.9	1.13	0.11	10.1	–0.035	(–0.132 to 0.062)	0.078	>0.10
3.0 to 4.0	38	3.2	1.11	0.12	10.5	0.003	(–0.153 to 0.16)	0.006	>0.10
3.0 and over	52	3.4	1.11	0.10	9.4	–0.012	(–0.049 to 0.03)	0.092	>0.10

Abbreviations are as under Tables 1 and 2.
[a]By ICP-MS (unit: μg/l).
[b]The ratio of Cd by GFAAS over Cd by ICP-MS.
[c]LOD=0.1 μg/l.

give about 16% greater value than ICP-MS, and that the difference in the results by the two analytical methods should be stabilized at higher Cd-B, e.g., Cd-B of 2 μg/l or greater (Table 4).

Discussion

Analytical principles for ICP-MS and GFAAS are quite different, measurement of mass of the selected isotope in the former method and that of light intensity after heat atomization of the element in the latter. In the present analyses, the standard addition method was employed in both analyses. It should be noted that the between-methods agreement may be influenced not only by the characteristics of the instrument employed and analytical conditions including the performance of the instruments, but also by the materials analyzed. Accordingly, allowance for the difference in analysis results between methods should be more generous than the case of traditional analysis for chemicals especially when a organics-rich complex matrix such as blood is subjected to the analysis. In practice, changes by $<\pm$ 20% was considered to be slight in the present study.

Analyses of the standard materials (Table 1) with Cd-B at two different levels, i.e., Level 1 at ca. 0.7 μg/l and Level 2 at ca. 6 μg/l, showed that GFAAS-based values were slightly greater (13 and 15% at Levels 1 and 2, respectively) than corresponding ICP-MS-based values. In this respect, the difference in the quality control standard values being lower for GFAAS than that for ICP-MS was quite contrary to the expectation. The recovery (an indicator of accuracy) was better for ICP-MS (93 and 101%) than for GFAAS (111 and 136%), whereas the CV (a precision indicator) was better for GFAAS (3.2 and 3.6%) than for ICP-MS (4.6 and 9.5%). It should be added that both experimental AM values (by ICP-MS and by GFAAS) were well within the 95% confidence intervals of the quality control standard values (Table 1). The LOD is lower for ICP-MS than for GFAAS but both are sufficiently low for Cd-B determination in practice. Thus, it was difficult to identify the superiority between the two methods, even though the equipment for ICP-MS is several times more expensive than that for GFAAS.

In the present survey, blood samples were collected from a large number of subjects (1,159 adult women) who had no occupational exposure to Cd. The participating subjects were solely from Japan, which may allow expectation that potential effects of ethnic life factors, if they are, should be minimal in applying the present study conclusion to Cd-B analysis among Japanese populations. The large number of cases made it possible to carry out detailed analysis of Cd-B level-dependent changes in compatibility of the results by two analysis methods (e.g., Fig. 3 and Table 4).

The present analyses taking Cd-B by ICP-MS as an independent variable and Cd-B by GFAAS as a dependent variable clearly demonstrated that Cd-B values as measured by the two methods correlate closely with each other ($r=0.964$, $p<0.01$). The slope, 1.059, was greater than 1.0 by 5.5% suggesting that the values by GFAAS would be slightly greater than the values by ICP-MS. Further analysis showed that the variations in the agreement in the results between the two methods were wider when Cd-B was smaller (e.g., $\leq$2 μg/l; Table 4) whereas the ratio of Cd-B by GFAAS over that by ICP-MS converged to a constant of 1.155 when Cd-B was e.g., >3 μg/l (Table 4).

As discussed above, literature survey showed that only two reports[7, 8] are available on possible difference in results on Cd-B between ICP-MS and GFAAS. Zhang *et al.*[7] analyzed 418 blood samples collected from residents (with no occupational exposure to Cd) in Japan and China (Cd-B being up to 3 μg/l as $GMxGSD^{1.65}$). GM Cd-B by ICP-MS was 83% of that by GFAAS. When log [Cd-B by GFAAS] and log [Cd-B by ICP-MS] were taken as an independent and a dependent variable, respectively, the slope of the regression line was 0.92 (0.78 for 198 Chinese cases and 0.96 for 220 Japanese cases), suggesting that ICP-MS gave lower values than GFAAS. Furthermore, the agreement was better for the one-third group of the highest Cd-B (88%) than the middle (82%) or the lowest one-third (81%). The findings as a whole are very similar to the present observation in the sense that the correlation is close between the two sets of measurements, that ICP-MS gives slightly lower values than GFAAS, and that the agreements are better for the cases with higher Cd-B than the cases with lower Cd-B. White[8] compared results of measurement of 90 samples for Cd-B (0.06 to 7 μg/l) by the two methods and found very close correlation between the paired values; the correlation coefficient was as high as >0.99 and the slope was 1.03. Although the slope may suggest that ICP-MS gave slightly larger values than GFAAS, no detailed statistical evaluation was reported, unfortunately.

Separate from blood or Cd, Ndung'u *et al.*[10] analyzed Pb in four wine vinegar samples by the two methods and found that ICP-MS gave smaller values than GFAAS (88 to 98%). Townsend *et al.*[11] in contrast found no difference between the results of analysis by the two methods when they measured Cd in eight water samples from a river. From literature survey, therefore, it may be possible to conclude that ICP-MS gives no higher values than GFAAS.

The observation on between-methods difference may carry practical implication that both methods can be used inter-convertibly in the Cd-B range around the biological exposure index (BEI) of 5 μg/l[3] or higher, but care should be practiced for the method-dependent difference when dealing with samples from general populations with low GM Cd-B levels, e.g., 0.28 to 0.71 μg/l[12–15] (the values are after conversion from AM to GM by the moment method[16] for uniformity in presentation). Nuttal *et al*[12], for example, obtained 0.71 μg/l Cd-B as GM by ICP-MS, and the level if it had been measured by GFAAS could be in excess of 0.8 μg/l, as values by ICP-MS may be lower by 15–16% than values by GFAAS (Table 3).

Conclusions

The results by the two methods for Cd-B, i.e., GFAAS and ICP-MS, correlate with each other closely enough to allow conversion when Cd-B is ≥2 μg/l. Care for the method-dependent difference may be necessary when Cd-B is 2 μg/l or lower as the results by GFAAS would be greater than the ICP-MS results by 15–16%.

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