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Immunochromatographic assay of cadmium levels in oysters

Kosuke Nishi [a,*], In-Hae Kim [a], Takaaki Itai [b], Takuya Sugahara [a,c], Haruko Takeyama [d], Hideo Ohkawa [e]

[a] Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
[b] Center for Marine Environmental Studies (CMES), Ehime University, 2-5 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[c] South Ehime Fisheries Research Center, Ehime University, 1289-1 Funakoshi, Ainan, Ehime 798-4292, Japan
[d] Department of Life Science and Medical Bioscience, Waseda University, 2-2 Wakamatsu-cho, Shinjuku, Tokyo 162-8480, Japan
[e] Integrated Institute for Regulatory Science, Waseda University, 2-2 Wakamatsu-cho, Shinjuku, Tokyo 162-8480, Japan

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ABSTRACT

Oysters are one of foodstuffs containing a relatively high amount of cadmium. Here we report on establishment of an immunochromatographic assay (ICA) method of cadmium levels in oysters. Cadmium was extracted with 0.l mol L^{-1} HCl from oysters and cleaned up from other metals by the use of an anion-exchange column. The behavior of five metals Mn, Fe, Cu, Zn, and Cd was monitored at each step of extraction and clean-up procedure for the ICA method in an inductively coupled plasma-mass spectrometry (ICP-MS) analysis. The results revealed that a simple extraction method with the HCl solution was efficient enough to extract almost all of cadmium from oysters. Clean-up with an anion-exchange column presented almost no loss of cadmium adsorbed on the column and an efficient removal of metals other than cadmium. When a spiked recovery test was performed in the ICA method, the recovery ranged from 98% to 112% with relative standard deviations between 5.9% and 9.2%. The measured values of cadmium in various oyster samples in the ICA method were favorably correlated with those in ICP-MS analysis (r^2=0.97). Overall results indicate that the ICA method established in the present study is an adequate and reliable detection method for cadmium levels in oysters.

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1. Introduction

Cadmium (Cd) is a highly toxic heavy metal for humans. When humans are exposed to Cd through diet or tobacco smoke, it is principally distributed into kidneys [1,2]. A long-term exposure to Cd causes renal disease, osteomalacia, and/or osteoporosis [3]. One of examples of mass Cd poisoning "Itai–itai desease", which was characterized as serious renal tubular dysfunction and osteomalacia, was caused by a long term intake of Cd-contaminated rice grown in Cd-polluted paddy field in Japan [4]. In addition, it was recently reported that Cd exposure increases cancer risks [5,6], and exposures even at lower levels induce detrimental effects on humans over time because of its long half-life in the body [1,7,8]. Therefore, Cd is a threat for human health, indicating that a thorough monitoring of Cd in foods and the environment is of great significance.

Determination of Cd in foods and environmental samples is usually performed in an instrumental analysis such as inductively coupled plasma-mass spectrometry (ICP-MS), ICP-atomic emission spectrometry, and atomic absorption spectrophotometry, which analyze Cd precisely and accurately. However, these methods are costly and laborious since analytical expertise and expensive equipments are required. As an alternative method for detection of Cd, an immunochemical assay has attracted attention because it is cost-effective and even as sensitive as the instrumental analysis. The immunochemical assay based on a high affinity and specificity of an antibody to an antigen is commonly used in not only medical diagnostics but also environmental analysis. Blake et al. [9] demonstrated that a Cd-specific antibody can be raised by immunizing animals with a stable Cd-chelate complex conjugated to a carrier protein. Thereafter, Sasaki et al. [10] reported on an immunochromatographic assay (ICA) for Cd by the use of a monoclonal antibody specific to the Cd-ethylenediaminetetraacetic acid (EDTA) complex. Recently, it was reported that the ICA detects Cd in a variety of food and environmental samples such as rice, eggplants, and soils with a high sensitivity [11–13]. However, it was found that the presence of the metals Mn, Fe, Cu, and Zn even at a low concentration after purification interferes with a stable detection of Cd in the ICA, resulting in relatively high standard deviations in measured values. Therefore, an anion-exchange column was used for separation of Cd from the other metals, since Cd forms tetrachlorocadmate ion and behaves as an anion in HCl solutions [14].

Abbreviations: EDTA, Ethylenediaminetetraacetic acid; ICA, Immunochromatographic assay; ICP-MS, Inductively coupled plasma-Mass spectrometry; RSD, Relative standard deviation; SD, Standard deviation.

* Corresponding author.
E-mail address: knishi@agr.ehime-u.ac.jp (K. Nishi).

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In general, shellfishes such as oysters, clams, and mussels contain various metals including Cd. Especially, oysters were reported to have higher levels of Cd and Zn [15,16]. Currently, an adequate method to detect Cd levels in oysters readily and inexpensively is not available. In the present study, we focused on establishment of a method of ICA useful for a stable and sensitive detection of Cd levels in oysters. Five metals including Cd were monitored by the use of ICP-MS at all steps of the procedure including extraction and clean-up for establishment of an efficient method of Cd detection in oysters. The Cd ICA method optimized in this work was highly accurate and precise for detection of Cd levels in a variety of oyster samples.

2. Experimental

2.1. Chemicals and biochemicals

Nitric acid (ultratrace analysis grade) and standard solutions of Cd, Cu, Fe, Mn, Rh, and Zn were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ultra pure water (18.2 MΩ) was prepared in a Milli-Q system (Millipore, Billerica, MA, USA). Standard reference material oyster tissue (SRM 1566b) was obtained from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

2.2. Cd ICA

An ICA kit for Cd detection (Cadmierre^{TM}) consisting of the anti-Cd-EDTA antibody labeled with gold particles, immunochromatography testers, anion-exchange columns, and the Cd-eluting solution was purchased from Sumika Chemical Analysis Service (Osaka, Japan). The procedure for Cd assay with the ICA kit was performed in accordance with the manufacturer's instruction. The quantification range of Cd by this ICA kit is from 0.01 mg L^{-1} to 0.06 mg L^{-1} according to the manufacturer's instruction.

2.3. Preparation of oyster samples for Cd ICA

Oysters cultured in Hiroshima, Japan in winter 2009 were purchased from a local market. The oysters were washed with deionized distilled water, and excess moisture was blotted on a paper towel. Approximately 100 g of oysters was pooled together and homogenized in a food processor to prepare a representative oyster sample. Aliquots of the homogenate were kept at -35 °C until use. This representative oyster sample was used to establish an assay method for Cd levels in oysters. Oysters cultured in three different areas, Hiroshima, Ehime, and Miyagi in Japan in winter 2010, were purchased from a local market to measure Cd levels in ICA. The oysters were individually washed with deionized distilled water and homogenized in a food processor, and then subjected to Cd ICA and ICP-MS analysis.

2.4. Preparation of oyster samples for ICP-MS analysis

All glassware and polypropylene plastic containers used for oyster samples were cleaned with a neutral detergent, soaked in 4 mol L^{-1} HNO_3 over 24 h, thoroughly rinsed with Milli-Q water, and dried. Each of oyster subsamples was weighed (10 g, wet weight), freeze–dried overnight, weighed again to measure a moisture content in the sample, and powdered. Around 0.2 g of the dried sample was added to a Teflon vessel, followed by adding 3.5 mL of HNO_3. Then, the mixture was decomposed in a microwave-assisted digestion system (Ethos D, Milestone Srl, Sorisole, BG, Italy). The microwave digestion program consisted of the following stages: 2 min at 250 W, 3 min at 0 W, 5 min at 250 W, 5 min at 400 W, 5 min at 500 W, 5 min at 400 W, and 5 min for ventilation and 2 min at 250 W, 3 min at 0 W, 5 min at 250 W, 5 min at 400 W, 10 min at 500 W, 10 min at 400 W, and 5 min for ventilation. After decomposition, vessels containing samples were allowed to cool down at room temperature for 30 min, and the digested samples were each diluted to 50 mL with Milli-Q water. Standard reference material oyster tissue (NIST SRM 1566b) was also decomposed in the same method as described above. The digested samples were each analyzed in an ICP-MS.

2.5. ICP-MS analysis

Concentrations of five selected metals (Mn, Fe, Cu, Zn, and Cd) were analyzed in an ICP-MS (Agilent 7500cx, Agilent Technology, Santa Clara, CA, USA; element m/z; Mn 55, Fe 57, Cu 63, Zn 66, Rh 103, Cd 111). Multielement standard solutions of Cd, Cu, Fe, Mn, and Zn were prepared by diluting standard stock solutions of the metals in 5% HNO_3 prior to use. Rh was used as an internal standard to monitor the variation due to instrument drift and/or matrix effects. Analytical accuracy in an ICP-MS was evaluated by analyzing the certified standard reference material oyster tissue. When non-digested samples were subjected to the instrument, dilution in 5% HNO_3 and filtration through a 0.45 μm polyvinylidene fluoride membrane filter (Whatman, Maidstone, UK) were performed prior to analysis. Each determination was done in three replicates. Relative standard deviation (RSD) of each determination was confirmed to be less than 3% by the use of RSD (%)= (SD/mean) × 100.

2.6. Extraction and clean-up of Cd from oysters

A 2 g portion of a homogenized oyster sample was placed in a cleaned polypropylene bottle, and 20 mL of 0.l mol L^{-1} HCl was added to the bottle. After a vigorous hand-shaking for 1 min, a portion of the suspension of the HCl extract was filtered through a qualitative filter paper (Qualitative no. 2, Advantec Toyo, Tokyo, Japan). Then, 1 mL of the filtrate was loaded on an anion-exchange column, which was designed on the basis of Akatsuka's method to concentrate and separate Cd from other metal ions [14]. After washing the column with 1 mL or 2 mL of 0.1 mol L^{-1} HCl, Cd was eluted by adding 1 mL of the Cd-eluting solution accompanied with the anion-exchange columns. The HCl extract and solutions derived from each step during clean-up of the representative oyster sample were analyzed by ICP-MS. Eluates from the columns were subjected to analysis of Cd in both ICP-MS and ICA.

2.7. Detection of Cd in ICA

Cd in cleaned-up eluates and standard solutions was measured in the ICA kit. Briefly, 20 μL of eluates from the anion-exchange columns or $CdCl_2$ standard solutions in 0.01 mol L^{-1} HCl was mixed with 380 μL of 0.1 mol L^{-1} Tris buffer solution containing 1.0 μmol L^{-1} EDTA to neutralize the solution and to form Cd-EDTA complexes. Then, 100 μL of the mixture was reacted with a freeze–dried anti-Cd-EDTA monoclonal antibody labeled with gold particles to yield antigen (Cd-EDTA)-antibody complexes. 75 μL of the solution was each loaded on a sample pad of an immunochromatographic tester. After incubating at room temperature for 30 min to 40 min, color densitometry of the red band appearing at the test line of the tester was read in a portable immunochromatography reader DiaScan 30-D (Otsuka Electronics, Osaka, Japan). Three measurements were conducted for each sample.

2.8. Spike and recovery test

Various amounts of Cd were added to a portion of the representative oyster sample by using a standard solution of $CdCl_2$ prepared in 0.01 mol L^{-1} HCl. Then, Cd levels spiked to the oyster sample were measured in ICA after extraction and clean-up with an anion-exchange column as described above. Recovery (%) was calculated based on total Cd concentrations. The measurement was done in triplicate.

3. Results and discussion

3.1. ICP-MS analysis of metals in the standard reference material oyster tissue

In order to establish an assay method of Cd levels in oysters by the use of the ICA kit, ICP-MS was employed to monitor not only Cd levels but also four metals Mn, Fe, Cu, and Zn in the samples, since those metals are generally present in oyster samples and the anti-Cd-EDTA antibody used in the ICA kit shows the cross-reactivity with such metal ions complexed with EDTA [10]. Therefore, monitoring of these metals is necessary to validate the accurate measurement of Cd levels in the ICA kit. At first, accuracy and precision of ICP-MS analysis were evaluated by measuring concentrations of the five metals in the standard reference material oyster tissue (SRM 1566b) from NIST. The standard reference material oyster tissue was acid-digested in a microwave and then subjected to ICP-MS analysis. As shown in Table 1, the concentrations of the metal ions observed between the certified and measured values were similar. The recovery (%) was ranged from 96% to 105% for Mn, Fe, Cu, Zn, and Cd with small variations. These results indicate that ICP-MS analysis is highly accurate for the measurement of the five metals in the oyster tissue. Since the measured values were in good agreement with the certified values, the microwave-assisted acid decomposition method followed by ICP-MS analysis was verified to be suitable for monitoring the metals in oyster samples.

3.2. Cd extraction with HCl from oyster samples

In order to measure Cd levels in oysters by the use of the ICA kit, a rapid and simple extraction of Cd from oyster samples is required. It has been known that a low concentration of HCl is effective to extract and recover Cd from a variety of environmental samples quantitatively, indicating that an HCl solution seems to be useful for extraction of Cd from oyster samples. So, it was attempted to extract Cd from the representative oyster sample prepared from oysters cultured in Hiroshima, Japan in winter 2009 by the use of 0.1 mol L^{-1} HCl. Also, acid decomposition with HNO_3 under microwave-assisted conditions was examined as described above. When the recovery of the metals was compared between acid digestion and HCl extraction as seen in Table 2, the amounts of the extracted metals with a solution of 0.1 mol L^{-1} HCl were comparable to those with the acid degradation except for Fe. Almost no loss was found in the concentration of Cd, suggesting that the HCl extraction is highly useful for extraction of Cd from the representative oyster sample. The low recovery of Fe from the oyster sample by the HCl extraction was consistent with the results from a seawater sample [14], indicating that a low concentration of HCl solution is also useful for separation of Cd from Fe in the extraction step. The concentrations of Mn, Fe, Cu, and Zn in the representative oyster sample were 41–730-fold higher than that of Cd. Although the monoclonal antibody used in the ICA kit is highly specific to the Cd-EDTA complex, it cross-reacts with Mn-EDTA, Fe-EDTA, Cu-EDTA, and Zn-EDTA at a level of 0.57%–1.4% as compared to 100% of Cd-EDTA [10]. Excess amounts of the co-existing metals may result in overestimation of Cd levels by the cross-reactions with the antibody and thus interfere with the accurate measurement of Cd levels in the ICA kit. Therefore, removal of Zn in a higher amount and other metal ions in the HCl extracts of the representative oyster sample is of great importance to ensure the accuracy of Cd ICA. Akatsuka et al. reported that a coated column of C_{18}-bonded silica gel with methyltricaprylylammonium chloride selectively concentrates Cd from water samples under acidic conditions [14]. In addition, clean-up of Cd from a couple of food extracts with the anion-exchange column was reported to be successful [11–13]. So, the performance of the anion-exchange column for clean-up of Cd from a mixture of metals in the HCl extracts of oysters seemed to be important for the next examination.

Table 1
Analysis of five metals in the standard reference material oyster tissues (NIST SRM 1566b) by ICP-MS.

	Metal concentration (mg kg^{-1} dry weight)[a]				
	Mn	Fe	Cu	Zn	Cd
Certified	18.5 ± 0.2	205.8 ± 6.8	71.6 ± 1.6	$1{,}424 \pm 46$	2.48 ± 0.08
Measured[b]	19.2 ± 0.3	198 ± 2	69.8 ± 0.3	$1{,}468 \pm 18$	2.60 ± 0.04
Recovery (%)	104	96	97	103	105

[a] Mean $\pm$ SD ($n=3$)
[b] Around 0.2 g each of the standard reference material oyster tissues was decomposed by the addition of 3.5 mL of HNO_3 under microwave-assisted digestion conditions, and then subjected to ICP-MS analysis.

3.3. Anion-exchange column chromatography for clean-up

The HCl extracts of the representative oyster sample were each filtered through filter papers. The filtrates were each loaded on anion-exchange columns. The columns were each washed with 1 mL of 0.1 mol L^{-1} HCl, and then adsorbed Cd was eluted with the Cd-eluting solution. The obtained HCl extracts, filtrates, flow-through fractions, wash fractions, and eluates were each subjected to analysis of five metals in ICP-MS. As shown in Table 3, concentrations of the five metals were decreased step by step during clean-up procedure without a loss of extracted Cd. Filtration to remove large debris in the HCl extract had no influence on the metal concentrations including Cd when compared to the HCl extract. A flow-through fraction, which flowed out of the column loaded with 1 mL of the filtrate, contained $>80\%$ of Mn, Fe, and Cu and approximately 50% of Zn as compared with the amounts in the filtrate or the HCl extract, while almost no Cd was found in the flow-through fraction. This indicates that Cd is completely adsorbed on the column, while the other metals are mostly flowed out without a strong retention on the column. In addition, the following washing fraction of the column with 1 mL of 0.1 mol L^{-1} HCl removed almost remained metals (Mn, Fe, Cu, and Zn) from the column without a loss of Cd. It is well known that Cd(II) easily forms a negatively charged tetrachlorocadmate ion in a solution of HCl. This formation of the complex may explain the selective isolation of Cd in the HCl extract on the anion-exchange column. The results in Table 3 indicate that almost all of Cd is recovered in the eluate of the column when compared to that in the HCl extract, suggesting that the anion-exchange column is highly useful for clean-up of Cd from co-existing metal ions in the HCl extract from the representative oyster sample, and that all steps performed during clean-up procedure do not lose the extracted Cd.

On the other hand, a little of metal ions other than Cd still remained on the column as indicated in the analysis of the metals in the eluate. In particular, the concentration of Zn was relatively

Table 2
Analysis of five metals in the representative oyster sample after acid decomposition or HCl extraction.

	Metal concentration (mg kg^{-1} wet weight oyster)[a]				
	Mn	Fe	Cu	Zn	Cd
Acid decomposition[b]	7.59 ± 0.05	23.1 ± 0.1	9.72 ± 0.04	132 ± 0	0.176 ± 0.003
HCl extraction[c]	7.46 ± 0.41	3.26 ± 0.59	9.13 ± 0.12	132 ± 1	0.181 ± 0.006

[a] Mean ± SD (n=3)
[b] A 0.2 g portion of a freeze-dried representative oyster sample was decomposed by the addition of 3.5 mL of HNO_3 under microwave-assisted digestion conditions, and then analyzed in ICP-MS.
[c] To a 2 g portion of a homogenized representative oyster sample was added 20 mL of 0.l mol L^{-1} HCl. After a vigorous hand-shaking for 1 min, a portion of the suspension of the HCl extract was diluted in 5% HNO_3 and filtrated through a 0.45 μm polyvinylidene fluoride membrane filter. Then, the filtrate was analyzed in ICP-MS.

Table 3
Analysis of five metals in each step of clean-up procedure of HCl extracts from the representative oyster sample in ICP-MS.

Step of clean-up	Metal concentration (mg L^{-1} solution)[a]				
	Mn	Fe	Cu	Zn	Cd
HCl extract[b]	0.746 ± 0.041	0.359 ± 0.059	0.913 ± 0.012	13.2 ± 0.1	0.018 ± 0.001
Filtrate[c]	0.763 ± 0.008	0.348 ± 0.033	0.940 ± 0.047	13.0 ± 0.2	0.018 ± 0.000
Flow-through[d]	0.636 ± 0.009	0.456 ± 0.018	0.728 ± 0.009	6.53 ± 0.13	< 0.001
Washing[e]	0.273 ± 0.018	0.171 ± 0.014	0.326 ± 0.015	7.13 ± 0.52	< 0.001
Eluate[f]	0.001 ± 0.000	0.017 ± 0.004	0.012 ± 0.002	0.056 ± 0.011	0.017 ± 0.003

[a] Mean ± SD (n=3).
[b] HCl extracts from the representative oyster sample.
[c] Filtrates after filtration of HCl extracts from the representative oyster sample through a filter paper.
[d] Flow-through fractions from anion-exchange column chromatography of 1 mL of the filtered HCl extracts from the representative oyster sample.
[e] Washing fractions from anion-exchange column chromatography with 0.l mol L^{-1} HCl after loading the filtered HCl extracts from the representative oyster sample.
[f] Eluates from anion-exchange column chromatography of the filtered HCl extracts from the representative oyster sample.

Table 4
Analysis of five metals in the eluates from the anion-exchange columns in ICP-MS.

Washing HCl volume (mL)[a]	Metal concentration (mg kg^{-1} wet weight oyster)[b]				
	Mn	Fe	Cu	Zn	Cd
1	0.01 ± 0.00	0.17 ± 0.04	0.12 ± 0.02	0.56 ± 0.11	0.17 ± 0.03
2	0.01 ± 0.00	0.16 ± 0.02	0.06 ± 0.05	0.24 ± 0.03	0.18 ± 0.00

[a] The HCl extract from the representative oyster sample was loaded onto the anion-exchange column. The column was washed with 1 mL or 2 mL of 0.1 mol L^{-1} HCl, and then eluted. The eluate from the column was subjected to ICP-MS analysis.
[b] Mean ± SD (n=3).

Table 5
Assay of Cd levels in the representative oyster sample in the ICA kit.

Washing HCl volume (mL)[a]	Cd level (mg kg^{-1} wet weight oyster)[b]
1	0.21 ± 0.03
2	0.18 ± 0.01

[a] The HCl extract from the representative oyster sample was loaded to the anion-exchange column. The column was washed with 1 mL or 2 mL of 0.1 mol L^{-1} HCl, and then eluted. The eluate from the column was subjected to Cd assay in the ICA kit.
[b] Mean ± SD (n=3).

high over those of the other metals, which may give a negative effect on the accurate measurement of Cd in the ICA kit. For further removal of the metals from the column prior to elution, the column was washed with 1 mL or 2 mL of 0.1 mol L^{-1} HCl. As shown in Table 4, no change in the concentrations of Mn, Fe, and Cd in the eluate was observed. However, the amounts of Cu and Zn were reduced by 50%, implying that washing of the column with the twice volume is effective for further removal of Cu and Zn metals without a loss of Cd adsorbed on the column.

3.4. Cd assay in the representative oyster sample by the use of the ICA kit

Continuously, it was attempted to assay Cd levels in the representative oyster sample by the use of the ICA kit. As shown in Table 5, a slightly higher level of Cd was determined in the ICA method than that in ICP-MS when the columns were washed with 1 mL of 0.1 mol L^{-1} HCl and a relatively large variation (RSD=13%) was observed. However, when the column was washed with 2 mL of 0.1 mol L^{-1} HCl, the measured Cd level in the ICA method was similar to that analyzed in ICP-MS with a smaller variation (RSD=6%). The results suggest that 1 mL of washing HCl volume is not enough to remove the remained metals on the column inducing cross-reactions with the antibody used in the ICA kit, which seemed to result in relatively low accuracy and precision of the detection. On the other hand, washing of the column with 2 mL of 0.1 mol L^{-1} HCl was optimal to reduce the interfering effect by the remained metals, leading to the improved accuracy and precision for Cd assay in the ICA kit. Overalls indicate that a stable measurement of Cd levels in the representative oyster sample in the ICA kit is attained from an enough elimination of metal ions, especially Cu and Zn, from the column.

The representative oyster sample was found to contain Cd at a level of 0.18 mg kg^{-1} wet weight oyster by the use of the ICA method (Table 5). A similar result was obtained in ICP-MS analysis (Table 2), implying that Cd assay in the ICA method established in this study is optimal for measurement of Cd in the representative oyster sample. In order to further validate Cd assay in the ICA method, known amounts of a standard solution of $CdCl_2$ were spiked to the representative oyster sample in a range of 0.20 mg kg^{-1} to 0.80 mg kg^{-1}, and then Cd concentrations were assayed in the ICA method. As seen in Table 6, the measured Cd concentrations in the ICA method were similar to the calculated Cd amounts, resulting in recovery percentage of 98%–112%

Table 6
Assay of Cd concentrations spiked to the representative oyster sample in the ICA kit.

Spiked Cd (mg kg^{-1} wet weight oyster)	Total Cd concentration in spiked sample[a] (mg kg^{-1} wet weight oyster)	Measured Cd concentration[b] (mg kg^{-1} wet weight oyster)	Recovery[c] (%)	RSD[d] (%)
0	0.18	0.18 ± 0.01	98	6.7
0.20	0.38	0.40 ± 0.04	104	9.0
0.40	0.58	0.61 ± 0.05	105	7.9
0.60	0.78	0.87 ± 0.08	112	9.2
0.80	0.98	1.01 ± 0.06	103	5.9

[a] The representative oyster sample originally contained Cd at 0.18 mg kg^{-1} wet weight as shown in Table 2. Known amounts of $CdCl_2$ solutions diluted in 0.01 mol L^{-1} HCl were added to the representative oyster sample. The spiked samples were each extracted with 0.1 mol L^{-1} HCl and then subjected to clean-up with the anion-exchange column chromatography. Cd levels were assayed in the ICA kit.
[b] Mean $\pm$ SD ($n=3$).
[c] Recovery (%)=(measured Cd level)/(total Cd level) $\times$ 100.
[d] RSD (%)=(SD/mean) $\times$ 100.

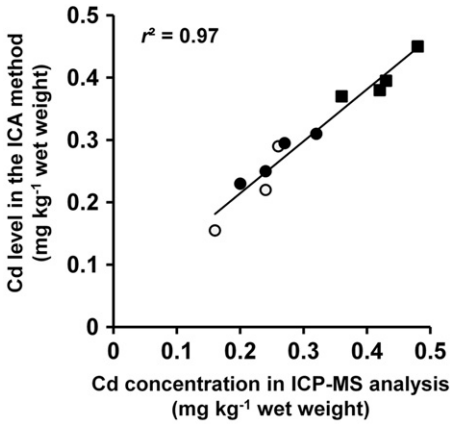

Fig. 1. Cd levels in a variety of oysters measured in both the ICA kit and ICP-MS analysis. Oysters cultured at Hiroshima, Ehime, and Miyagi in Japan were used. For ICP-MS analysis, the oysters were acid-digested. The equation of regression line; $y=0.83x+0.04$. $r^2=0.97$. ●; Oysters cultured at Hiroshima, ○; oysters cultured at Ehime, ■; oysters cultured at Miyagi.

with less than 9.2% of RSD. These results suggest that Cd assay in the ICA method established in this study is useful for a stable detection of Cd in various concentrations in oyster samples.

3.5. Cd levels of various oyster samples in both the ICA kit and ICP-MS

Since Cd assay in the ICA method established in this study was useful for accurate and precise detection of Cd levels in oysters, it was further attempted to measure Cd levels in oysters cultured in three different areas in Japan in both the ICA kit and ICP-MS. Fig. 1 shows that the Cd levels in these oysters were ranged between 0.1 mg kg^{-1} and 0.5 mg kg^{-1} wet weight in both the ICA kit and ICP-MS. Relatively high levels of Cd were found in the oysters cultured in Miyagi compared with those cultured in Hiroshima or Ehime. The results also exhibited a high correlation ($r^2=0.97$) between Cd assay in the ICA method and ICP-MS analysis. These results suggest that the optimized ICA method established in this study is highly useful for assay of Cd levels in a variety of oyster samples with high accuracy and precision.

4. Conclusions

This work focused on establishment of Cd assay in oysters in the ICA method. The concentrations of five metals including Cd in each step of clean-up procedure of the HCl extract of the representative oyster sample were monitored by ICP-MS to evaluate the overall efficacy. The extraction of Cd in the oyster sample with 0.1 mol L^{-1} HCl provided a quantitative yield comparing to the results in ICP-MS analysis, suggesting that 0.1 mol L^{-1} HCl is optimal for extraction of Cd from the oyster sample. On the other hand, the extraction exhibited the presence of metals such as Mn, Fe, Cu, and Zn in 41–730-fold higher concentrations than that of Cd in the oyster sample. For clean-up of Cd from these metals, an anion-exchange column was used, which was most important for producing reliable detection results in the ICA method. Successful elimination of the metal ions was obtained without a loss of Cd concentration in the column chromatography. Washing of the column with 2 mL of 0.1 mol L^{-1} HCl was more effective than with 1 mL of washing to remove remained metals except for Cd from the column, leading to a further reduction in Cu and Zn levels by a 2-fold. This reduction resulted in a stable detection of Cd with a small variation in the ICA method. When various concentrations of Cd spiked to the oyster sample were analyzed by the optimized Cd assay in the ICA method, high recoveries with small variations were obtained. Furthermore, application of the method to various oyster samples provided highly correlated results with those in ICP-MS analysis. Overall results suggest that Cd assay in the ICA method established in this study is useful for accurate measurement of Cd concentrations in oysters without interfering effects of co-existing metals. This method can be used as a simple and quick detection tool to monitor Cd concentrations in oysters on site where expensive facilities like ICP-MS for the detection are not available.

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