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Selective Determination of Inorganic Arsenic (III), (V) and Organic Arsenic in Biological Materials by Solvent Extraction-Atomic Absorption Spectrophotometry

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A new method for the selective determination of inorganic arsenic (III), (V) and organic arsenic has been performed. Each chemical form of arsenic in biological materials was solubilized in 6 N hydrochloric acid. Inorganic arsenic (III) was extracted into toluene from 9 N hydrochloric acid solution, and inorganic arsenic (V) was also extracted after reduction by potassium iodide. Organic arsenic was retained in hydrochloric acid solution. After arsenic in toluene solution was back-extracted into water, aqueous or hydrochloric acid solution containing arsenic was wet digested with a mixture of HNO_3, H_2SO_4 and $HClO_4$, and arsenic was determined by atomic absorption spectrophotometry. The proposed method was applied to some kinds of algaes, shark muscle and orchard leaves (NBS SRM 1571). 90~100% of arsenic in algaes (*Wakame, Arame, Kazime*) and shark muscle was organic, while all the arsenic in orchard leaves was inorganic.

It is well known that the arsenic contents of marine animals and plants are much more than those of terrestrial animals and plants. Therefore, for the many people who eat a lot of marine fish and algaes, the high contents of arsenic in them are a very important problem. However, the potential toxicity of arsenic is considered to depend on its chemical form. Toxic aspects of arsenic have recently been reviewed by Frost.[1] In this review, the wide differences in the toxicity of the different chemical forms of arsenic are emphasized. Generally, inorganic arsenic is more toxic than the organic form, and trivalent arsenicals are most toxic. In relation to its toxicity, it is very necessary to develop methods to determine each chemical form of arsenic. Lunde[2] separated inorganic arsenic from organic arsenic by distillation as arsenic trichloride from 6.6 N hydrochloric acid solution, and reported that organic arsenic was more predominant than inorganic arsenic in marine animals and plants. However, the experimental conditions are rather severe and are not suited for analysis of many samples. Aggett and Aspell[3] separated inorganic arsenic (III) and (V) in the partially decomposed sample solution by adjusting the acidity of sample solution and by using sodium borohydride as reductant. They applied the method to certain plant materials and water. However, they did not mention organic arsenic. The APDC-MIBK extraction method in which inorganic arsenic (III) is separated from inorganic arsenic (V) was also reported by Kamada,[4] and applied to the determination of waste water.

We have remarked that inorganic arsenic (III) was extracted selectively from hydrochloric acid solution (above 8 N) with benzene,[5,6] and investigated the best procedure which could separate simultaneously inorganic arsenic (III), (V) and organic arsenic by solvent extraction. As a result, a new selective solvent extraction procedure has been developed, which separates inorganic arsenic (III), (V) and organic arsenic by toluene extraction and arsenic is determined by arsine generation—flame atomic absorption spectrophotometry.

REAGENTS AND APPARATUS

Reagent. Reagents were of AR or MAR grade and deionized water which had previously been distilled was used.

Commercial standard solution of sodium arsenite, 1000 ppm, for atomic absorption spectrophotometry (Kanto Kagaku Co.) was used as standard inorganic arsenic (III).

Standard inorganic arsenic (V) stock solution, 100 ppm, was prepared by dissolving 0.208 g of disodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) in 500 ml of water.

Standard organic arsenic stock solution, 100 ppm, was prepared by dissolving 0.145 g of *p*-arsanilic acid ($NH_2 \cdot C_6H_4 \cdot AsO(OH)_2$) in 10 ml of 20% sodium carbonate aqueous solution, acidifying with 10 ml of 10% sulfuric acid, and diluting to 500 ml with water.

Aliquots of these solutions were diluted to give the required concentration before use.

Potassium iodide solution (50 w/v%) was prepared by dissolving 50 g of potassium iodide (KI) in 100 ml of water, and the solution was stored in a brown-colored bottle.

Apparatus

a) Carbon-furnace atomic absorption spectrophotometry. A Varian Techtron Model 63 CRA carbon rod atomizer mounted in a Varian Techtron Model AA-1000 atomic absorption spectrophotometer was used. The tubular furnaces coated with pyrolytic graphite (3 mm in diameter, 9 mm in length) were modified by removing the coating. The monochrometer was set at 193.7 nm, the resonance line of arsenic, and the spectral slit width was 2.0 nm. A photomultiplier tube (Hamamatsu TV Co.: R106UH) was used as the detector.

Model 63 CRA carbon rod atomizer settings were: drying 40 seconds at 105°C; ashing 30 seconds at 105°C and atomizing 3 seconds at 2100°C. Nitrogen was used as furnace purge gas, and the flow-rate was 4 liter/min.

b) Flame atomic absorption spectrophotometry. A Seiko SAS-721 atomic absorption spectrophotometer equipped with an R106 photomultiplier tube (Hamamatsu TV Co.) and a water cooled 10 cm slot burner was used. It was connected to a Nippon Jarrel-Ash ASD-1A arsine generator. The flow-rate of argon and hydrogen were 10 liter/min and 3 liter/min, respectively. The flow-rate of auxiliary argon through the arsine generator was 5 liter/min. Observation height above the burner was 8 mm. The monochrometer was set at 193.7 nm and the spectral slit width was 1.0 nm.

An arsenic hollow-cathod lamp (Hamamatsu TV Co.) was used as light-source and was operated at 8 mA in both flame and furnace experiments.

PROCEDURE

Separation procedure to determine optimum extraction conditions. Take 10 ml of various concentration (3~12 N) of hydrochloric acid containing 100 μg of arsenic (each chemical form) in a separating funnel, with or without adding 1 ml of 20 or 50% potassium iodide aqueous solution, mix well by swirling, and stand for 15 min. Shake the funnel for 2 min with 10 ml of an organic solvent (benzene or toluene), and stand to separate the organic solvent.

Transfer 10 μl of the organic solvent with micropipette to the carbon-tube atomizer, and measure the absorbance at 193.7 nm under the operational conditions of the carbon-furnace atomic absorption spectrophotometry.

Separation procedure to determine arsenic. Take 10 ml of concentrated hydrochloric acid solution containing arsenic in a separating funnel, with or without adding 0.5 ml of 50% potassium iodide aqueous solution, mix well by swirling, and stand for 15 min. Shake the funnel for 2 min with 10 ml of an organic solvent (benzene or toluene) and stand to separate the organic solvent. Transfer the hydrochloric acid solution (lower layer) to a flask. Back-wash the organic solvent with 10 ml of the same concentration of hydrochloric acid, with or without 0.5 ml of 50% potassium iodide aqueous solution, by shaking for 1 min, and transfer the hydrochloric acid solution (lower layer) into the flask containing the previous hydrochloric acid solution. Back-extract inorganic arsenic from the organic solution (upper layer) into 10 ml of water by shaking for 1 min, and transfer the aqueous solution to a new flask.

Take 10 ml of hydrochloric acid solution (containing organic arsenic) or aqueous solution (containing inorganic arsenic) into a 100 ml conical flask, decomposed by wet digestion, and measure arsenic by arsine generation—flame atomic absorption spectrophotometry.

Wet digestion.[7] Place previously weighed sample (less than 1 g) or sample solution (less than 10 ml) into a 100 ml conical flask, add 10 ml of concentrated nitric acid and cover with a watch glass. Heat the mixture gently on a hot plate. Cool the mixture after subsidence of foaming, and add 1 ml of concentrated sulfuric acid and 2 ml of perchloric acid (60%). Heat the mixture again strongly on the hot plate to steady boiling for 1~1.5 hr. Add immediately a small amount of concentrated nitric acid to prevent analyte loss if slight charring occurs. Boil off perchloric acid, and concentrate residual sulfuric acid to less than 1 ml. Wash the residue into a 25 ml volumetric flask with 3 N hydrochloric acid after cooling. Determine arsenic by arsine generation–flame atomic absorption spectrophotometry.

Arsine generation–flame atomic absorption spectrophotometry.[7,8] Take appropriate volume of sample decomposed solution containing less than 1 μg of arsenic into a 100 ml reaction vessel (Nippon Jarrel-Ash Co.), and add 3 N hydrochloric acid to adjust the mixture volume to 25 ml. Add 1 ml of 20 w/v% potassium iodide aqueous solution and 0.5 ml of 20 w/v% stannous chloride concentrated hydrochloric acid solution, and stand for 15 min after well swirling. Set the reaction vessel in an arsine-generator, add about 0.8 g of zinc powder (arsenic free) enclosed with a medical wafer into the mixture, joint immediately the reaction vessel to the gas-reservoir, and stir the mixture magnetically. Introduce the AsH_3 and H_2 which were generated and reservoired into the Ar-H_2 flame in the flame atomic absorption spectrophotometer by the aid of the flow of auxiliary argone when the gas pressure in the gas-reservoir reaches 0.5 kg/cm^2, and measure the absorbance at 193.7 nm.

RESULTS AND DISCUSSION

Selection of extractants

Benzene and toluene were suitable as extractants. As there was no difference in extractability between them, toluene was adopted because of its low acute toxicity.

Extraction behaviour of each chemical form of arsenic

The extraction behaviour of each chemical form of arsenic is shown in Fig. 1. *Ca.* 85% of inorganic arsenic (III) was extracted from hydrochloric acid solution above 9 N, whereas inorganic arsenic (V) and organic arsenic were not extracted into toluene. On the addition of potassium iodide, the extraction rate of inorganic arsenic (III) and (V) increased to *ca.* 100% from hydrochloric acid solution above 7 N, but organic arsenic was not extracted. It was considered that inorganic arsenic (V) was reduced to arsenic (III) by potassium iodide and extracted into toluene. Furthermore, the extraction rate of inorganic arsenic (III) was increased by addition of potassium iodide. Inorganic arsenic (III) was considered to be extracted into toluene as arsenic trichloride (bp 130.21°C) from hydrochloric acid solution, and as arsenic triiodide (bp *ca.* 400°C) after addition of potassium iodide, because in the former case a significant amount of arsenic was lost with ashing temperature of 170°C in the carbon-furnace atomizer, whereas arsenic extracted from hydrochloric acid solution containing potassium iodide was retained for ashing temperature of about 240°C.

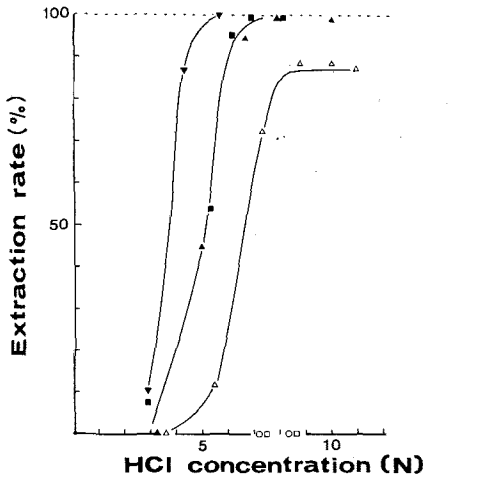

FIG. 1. Effect of Hydrochloric Acid Concentration on the Extraction Rate of Inorganic As (III), (V) and Organic As with Toluene.

△, inorganic As (III) from HCl; ▲, inorganic As (III) from (HCl+0.1 M KI); ▼, inorganic As (III) from (HCl+0.43 M KI); □, inorganic As (V) from HCl; ■, inorganic As (V) from (HCl+0.1 M KI); ○, organic As from HCl or (HCl+0.1 M KI).

The influence of the hydrochloric acid concentration on the reduction of inorganic arsenic (V) to arsenic (III) by potassium iodide was not observed in the range of 5~9 N.

Extraction rate of each chemical form of arsenic into toluene

In the measurement of extraction rate of inorganic arsenic (III) and (V) into toluene from hydrochloric acid solution, 2.5 μg and 25 μg of arsenic respectively in 10 ml of 9 N hydrochloric acid were used. In the case of inorganic arsenic (V) and organic arsenic in hydrochloric acid solution containing potassium iodide, the respective amounts of arsenic were 2.5 μg and 25 μg, and 0.5 ml of 50% potassium iodide aqueous solution was added to 10 ml of 9 N hydrochloric acid. Ten ml of toluene was used for extraction in both cases. Before the experiment, 0.1 g of L-ascorbic acid was added to inorganic arsenic (III) solution

to prevent interference by inorganic arsenic (V). Inorganic arsenic (V) solution was used after addition of 1 ml of 30% hydrogen peroxide to prevent interference by inorganic arsenic (III). As shown in Table I, the extraction rate of arsenic (III) into toluene from hydrochloric acid solution without the addition of potassium iodide is 85%. However, almost 100% of inorganic arsenic (III) was extracted when the extraction procedure was repeated 3 times.

TABLE I. EXTRACTABILITY OF ARSENIC INTO TOLUENE FROM HYDROCHLORIC ACID SOLUTION

Chemical form of arsenic		Compound	Hydrochloric acid solution 9 N HCl	9 N HCl, 0.13 M KI
Inorganic	As (III)	sodium arsenite	$D = 5.79$, $E = 85.3\%$	
	As (V)	disodium arsenate	$D = 0.016$, $E = 1.6\%$	$D = 65.84$, $E = 98.5\%$
Organic As		*p*-arsanilic acid		$D = 0.04$, $E = 3.9\%$

D: Distribution ratio,

$$D = \frac{W_{tol}/V_{tol}}{(W - W_{tol})/V_{HCl}}$$

W : Weight of arsenic added
W_{tol} : Weight of arsenic in toluene solution
V_{tol} : Volume of toluene
V_{HCl} : Volume of HCl solution (with or without KI)
E: Extraction rate $V_{tol}/V_{HCl} = 1$

Recommended selective determination procedure

On the basis of the results described above, the following procedure was proposed.

a) Inorganic arsenic (III) determination. Ten ml of sample solution was placed in the separating funnel. An appropriate amount of 12 N hydrochloric acid was added to adjust the acid strength of the sample solution to 9 N. Inorganic arsenic (III) was extracted by shaking for 2 min with 10 ml of toluene and this procedure was repeated twice. The total toluene solution (30 ml) was washed with 10 ml of 9 N hydrochloric acid. Inorganic arsenic was back-extracted from the 30 ml of toluene solution into aqueous solution (20 ml) using two 10 ml portions of water. Ten ml of aqueous solution was placed in a conical flask, decomposed by wet digestion, and arsenic was determined by arsine generation–flame atomic absorption spectrophotometry.

b) Inorganic arsenic (V) determination. After the extraction of inorganic arsenic (III), the hydrochloric acid solutions containing inorganic arsenic (V) and organic arsenic were combined, 1.5 ml of 50% potassium iodide aqueous solution was added and the solution was allowed to stand for 15 min in order to reduce arsenic (V) to (III). Reduced arsenic was extracted with 10 ml of toluene (three times). The toluene solution (30 ml) was washed with 10 ml of 9 N hydrochloric acid containing 0.5 ml of 50% potassium iodide aqueous solution. The arsenic was back-extracted from the 30 ml of toluene solution to water (20 ml) as above. Ten ml of aqueous solution was placed in a conical flask, decomposed by wet digestion, and arsenic was determined by arsine generation–flame atomic absorption spectrophotometry.

c) Organic arsenic determination. The hydrochloric acid solutions of the previous processes which contained organic arsenic were combined. Ten ml of hydrochloric acid solution was placed in a conical flask, organic arsenic was decomposed by wet digestion, and determined by arsine generation–flame atomic absorption spectrophotometry.

Application to biological materials

a) Procedure to determine optimum hydrochloric acid concentration. Before the solvent extraction process, it is necessary to solubilize each chemical form of arsenic in biological materials without any change. The partial decomposition method using 1 : 2 diluted nitric acid–sulfuric acid has been proposed by Aggett and Aspell.[8] However, this method could not be applied in the determination of organic arsenic, because the organic arsenic was decomposed to the inorganic form. Otherwise, in the determination of arsenic in soils, solubilization of arsenic by hydrochloric acid is commonly used as a pre-treatment.[9] Therefore, a study to determine whether each form of arsenic, particularly the organic one, could be solubilized by steeping biological materials in

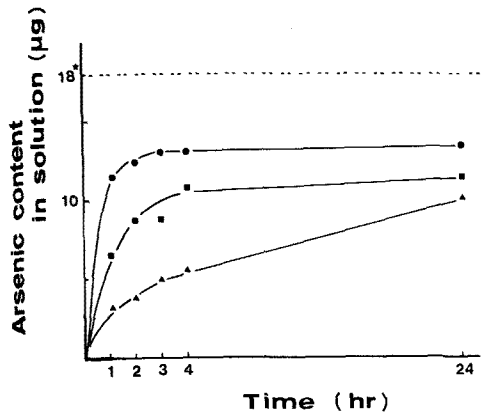

FIG. 2. Effect of Hydrochloric Acid Concentration on Solubilization of Arsenic to Hydrochloric Acid Solution from Biological Material.

●, 6~9 N HCl; ■, 3 N HCl; ▲, 1 N HCl.
Sample; *Undaria pinnatifida* 5.0 g (fresh weight).
Solution; 100 ml of HCl.
* Total arsenic content in 5.0g of *Undaria pinnatifida*.

hydrochloric acid was performed. At first, 5 g (fresh weight) of *Undaria pinnatifida* (Japanese name: *Wakame*) was placed in 100 ml of various concentrations of hydrochloric acid, and the total arsenic which was solubilized in hydrochloric acid as a function of time was measured. As shown in Fig. 2, arsenic was solubilized gradually in 1~3 N hydrochloric acid, whereas in 6~9 N hydrochloric acid the amount of solubilized arsenic reached plateaus within 2~3 hr. As a result, the procedure to solubilize arsenic in biological materials was decided as follows: Take 5~10 g of homogenized wet sample, or 1~2 g of powdered dry samples in a 100 ml beaker, add 50 ml of 6 N hydrochloric acid, and stand for one hour with occasional swirling. Filter the solution and collect the filtrate in a 100 ml beaker. Add again 50 ml of 6 N hydrochloric acid to the residue, and repeat the procedure to obtain 2nd and 3rd filtrates. The results of arsenic solubilization in biological samples using 6 N hydrochloric acid are shown in Table II. In shark muscle and orchard leaves, arsenic was almost completely solubilized after the 1st and 2nd filtrate. However, it was found that in other samples there was insolubilized arsenic,

TABLE II. SOLUBILIZATION OF ARSENIC IN SAMPLES BY 6 N HCl

	Total As[f] content (μg/dry weight g)	Arsenic content (μg/dry weight g) in filtrate 1st	2nd	3rd	in residue
Shark muscle[a]	60.4	56.1	3.2	0	0.1
Orchard leaves[b]	10.8	8.6	2.3	0.4	0.7
Undaria pinnatifida[c,e]	16.4	11.6	1.9	0.5	4.7
Ecklonia cava[d,e]	61.5	36.3	7.8	—	12.1
Eisenia bicyclis[d,e]	58.5	51.8	5.2	0.5	4.7
Hizikia fusiforme A[c]	93	66	8.6	—	9.1
Hizikia fusiforme B[d,e]	82.2	65.1	10.3	—	7.5

[a] Pulverized and mixed after lyophilization.[11] Shark (*Squalus mitsukurii*) was purchased at Choshi market.
[b] NBS-SRM 1571 (certified value of arsenic, 10±2 μg/g)
[c] Purchased at a market.
[d] Collected in Aburatsubo bay.
[e] Wet sample was used for experiment.
[f] Total arsenic contents were determined after samples were wet digested directly.

which was considered to be bound strongly to tissue.

b) Inorganic arsenic (III), (V) and organic arsenic in biological materials. The results of recovery test are shown in Table III. In shark muscle, orchard leaves, and *Undaria pinnatifida*, the recovery of inorganic arsenic (III) was *ca.* 85%, and the recovery of inorganic arsenic (V) was 90~99%. Low recovery of inorganic arsenic (III) might be caused by adsorption of arsenic to the emulsion in the first toluene extraction from the hydrochloric acid extract solution of biological material. Generally, however, satisfactory results were obtained for the recovery test. It is considered that there was little interference in the separation procedure by coexisting substances. However, in *Ecklonia cava* (Japanese name: *Kazime*) and *Hizikia fusiforme* (Japanese name: *Hiziki*), the recovery of inorganic arsenic (III) was 170~200%. When the toluene extraction was performed from the hydrochloric acid solution of these samples, the toluene solution was colored pink indicating the co-extraction of iodine.

Ordinally, the algaes contain about 0.4~0.6% of iodine on a dry weight basis.[10] It is well known that iodide ion reduces inorganic arsenic (V) to arsenic (III). For the determination of iodine in algaes, the colorimetric method by Ishibashi[12] was used with a modification using dry-ashing at 450°C and toluene as an extractant. The results were shown in Table IV. Various concentrations of potassium iodide were added to 2 ppm of inorganic arsenic (V) in 9 N hydrochloric acid solution to study the interference. The co-existence of 0.5 ppm, 1 ppm and 12 ppm iodide reduced 8.5%, 15.5% and 47.1% of inorganic arsenic (V) to arsenic (III), respectively.

The results of selective determination are shown in Table IV. Almost all the arsenic in shark muscle existed as the organic form. In contrast, all the arsenic in NBS orchard leaves was inorganic. Therefore, arsenic of 10 ppm level which NBS orchard leaves contains is considered not to be taken into its body, but to be a contaminant from an external source (*e.g.* pesticide like lead arsenate). However, arsenic of 0.01~0.1 ppm level which terrestrial animals and plants contain is considered to be taken into their bodies, we have not studied yet if arsenic of those is inorganic or organic because of their low concentrations. The inorganic arsenic (III) contents in NBS orchard leaves were in good agreement with the results by Aggett and Aspell.[3] *Undaria pinnatifida* contained about 10% of inorganic arsenic, with a slightly larger amount of inorganic arsenic (V) than inorganic arsenic (III). *Eisenia bicyclis* (Japanese name: *Arame*) contained

TABLE III. RECOVERIES OF AS (III) AND AS (V) IN HCl EXTRACTS OF BIOLOGICAL MATERIALS

Sample	Sample[a] (dry weight g)	As (III) (μg) added	As (III) (μg) found	As (V) (μg) added	As (V) (μg) found	Org As found (μg)	Recoveries (%) As(III)	Recoveries (%) As(V)
Shark muscle	1.0	(a)[b] —	0	—	0.06	5.17		
		(b) 5.0	4.21	5.0	4.53	5.18	84.2	89.4
Orchard leaves	2.0	(a) —	0.92	—	1.11	0		
		(b) 5.0	4.99	5.0	6.07	0	81.2	99.2
Undaria pinnatifida	1.66	(a) —	0.08	—	0.14	2.17		
		(b) 5.0	4.34	5.0	4.95	1.91	85.2	96.2
Ecklonia cava	1.43	(a) —	0.16	—	0.72	4.27		
		(b) 5.0	10.05	5.0	2.15	4.50	197.2	28.6
Hizikia fusiforme A	2.0	(a) —	17.65	—	2.43	7.40		
		(b) 5.0	26.20	5.0	3.99	7.72	170.9	31.2

[a] Each 50 ml of 1st, 2nd and 3rd filtrate were prepared.

[b] (a) Five ml of 1st filtrate and 5 ml of 2nd filtrate were combined for analysis. Third filtrate was not combined, because it had little arsenic.
(b) Five μg of As (III) and 5 μg of As (V) were added together to combined filtrates (5 ml of 1st filtrate and 5 ml of 2nd filtrate).

TABLE IV. CONTENTS OF INORGANIC AS (III), (V), ORGANIC AS AND IODINE IN VARIOUS BIOLOGICAL MATERIALS (ON DRY WEIGHT BASIS)

Sample		Total As content (μg/g)	Each form content of As(μg/g) in HCl extract solution: inorganic As(III)	inorganic As(V)	organic As	Residual As in sample (μg/g)	Iodine (%)
Shark muscle		60.4	0	0.78	59.49	0.05	—
Orchard leaves		10.8	4.69[a]	5.63	0	0.69	—
Undaria pinnatifida		16.4	0.48	0.87	12.37	4.73	<0.006
Ecklonia cava		61.45	6.15		29.83	12.12	0.201
Eisenia bicyclis		58.55	2.07		65.28	4.66	0.195
Hizikia fusiforme	A	93	50.21		18.06	9.08	0.072
	B	82.19	47.05		23.01	7.74	—

[a] 4.90 μg/g (value reported by Aggett and Aspell[3]).

about 5% of inorganic arsenic. *Ecklonia cava* also contained about 10% of inorganic arsenic. However, it was impossible to separate inorganic arsenic (III) and (V) in *Eisenia bicyclis, Ecklonia cava* and *Hizikia fusiforme,* because of iodide ion interference. In *Hizikia fusiforme,* the existing pattern of arsenic was different from those of the other algaes. More than 50% of total arsenic existed as the inorganic form. However, it is considered that the organic arsenic in *Hizikia fusiforme* may be partially converted to the inorganic form during sample solution preparation or separation. To check this point, it would be necessary to develop a new selective determination procedure.

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