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Arsenic Speciation in Fish on the Market

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ABSTRACT

Arsenic speciation in fish samples on the market was analyzed using gradient anion exchange high performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICPMS) detection. Freeze-dried samples were extracted by methanol and water mixture (8:2 v/v) with a high-speed Soxhlet system; the concentration of arsenobetaine (AsB), dimethylarsinic acid (DMAA), monomethylarsonic acid (MMAA), arsenate (As(V)) and arsenite (As(III)) in the extract were then determined using HPLC-ICPMS. The method for determining arsenobetaine concentration was confirmed using standard reference material (SRM) of BCR 627 (tuna fish) and DORM-2 (dogfish muscle); recovery rates were 95 and 90%, respectively. The arsenic compounds in 60 market-ready fish muscle samples were investigated. The predominant arsenic compound found in samples was AsB. Arsenate was detected in low concentrations (ranged in 0.02–0.34 mg/kg As for fresh weight), whereas DMAA, MMAA and arsenite content were undetectable. Average AsB content in cephalopods, small fish and large fish were 5.42, 1.57 and 1.54 mg/kg As (fresh weight), respectively. The weekly intake of As was calculated based on the consumption of fish by Taiwanese residents. The calculated results demonstrated that the intake is 30.6 μg/kg (total As) body weight/week, higher than the acceptable weekly intake of 15 μg/kg body weight/week for inorganic arsenic that was suggested by WHO. However, around 87% of As in fish muscle was AsB. AsB is non-toxic and non-carcinogenic to humans, and is rapidly excreted after ingestion. Therefore, intake of fish muscle is low risk based on investigation results.

Key words: Arsenic speciation, fish, arsenobetaine, weekly intake

INTRODUCTION

Arsenic (As) is a non-essential element to both humans and plants[1,2]. Toxicity of As greatly depends on its chemical form or "species", with inorganic arsenic more toxic than organic arsenic. The toxicities of arsenics from high to low are as follows: arsenite (As(III)) > arsenate (As(V)) > organic arsenic[3,4]. The chronic effects of As to human health include vascular disorders, leukopenia, anemia, black foot disease, skin cancer, and lung cancer[5-8].

Arsenic enters the human body primarily through (1) inhalation of smoke from smelters or burned fuel, (2) drinking water, (3) and food (seafood mainly)[9,10]. On the other hand, arsenic enters the aquatic environment via natural or anthropogenic pollution. Marine organisms for human consumption, such as fish and mollusks, can accumulate high concentrations of arsenic from water and food chains. The concentration of As in marine organisms ranges from 1–100 mg/kg (dry weight). The predominant arsenic compound in muscle tissues in these organisms is arsenobetaine (AsB)[11], which is nontoxic and non-carcinogenic to mammals. Moreover, AsB is excreted easily after being ingested by mammals, and the half-life of AsB in humans is 6 hours[12-16]. Although arsenobetaine predominates in the arsenic component to nearly all marine animals, other forms of arsenic like arsenochlorine, arsenosugars, tetramethylarsonium ion and trimethylarsine are sometimes found [11,14].

Previous studies have determined the heavy metal content in fish[17] and calculated the weekly intake of consumers of heavy metals from fish. Calculated results demonstrated that intake of As (total As) from fish was higher than the acceptable weekly intake of 15 μg/kg body weight/week for inorganic arsenic suggested by the WHO[18]. Survey of arsenic species in fish and assessing the safety of fish intake is necessary for human health reasons.

Methanol and water mixture has been used for extracting arsenic species from fish samples[19]. According to the report of Pizarro *et al.* (2003), stability of the arsenic increased as methanol content in the mixture increased[19]. Extraction techniques of centrifugal extraction[20], microwave-assisted extraction[21,22], accelerated solvent extraction and enzymatic digestion combined extraction[23,24], have been employed in other studies. The Soxhlet extraction system was utilized to reduce both solvent consumption and time needed for extraction. Numerous techniques have been developed for arsenic speciation in the extracts from biological samples[25-27].

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An analytical method capable of selectivity and sensitivity is required. The HPLC combined with atomic absorption spectrometry (AAS) has been applied for As speciation. This method is very complex and requires a hydride generation process following speciation of arsenic compounds and not all arsenic species form hydrides[23,27]. Notably, HPLC combined with ICPMS detection is increasingly used due to its interface simplicity and sensitivity of ICPMS[22, 28,29].

This study investigates the levels and speciation of arsenic in fish sample collected from local markets and supermarkets located in central Taiwan. In addition, safety of eating these fish was evaluated.

MATERIALS AND METHODS

I. *Reagents and Materials*

All solutions were prepared with de-ionized water (18.2 MΩ cm) purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Analytical reagent-grade chemicals and gradient-grade methanol from Merck (Darmstadt, Germany) were utilized. The AsB standard solution (BCR 626, 1031 mg/L based on As) was purchased from the Institute for Reference Materials and Measurements (Brussels, Belgium). The As(III), As(V), MMAA (monomethylarsonic acid) and DMAA (dimethylarsinic acid) standards were prepared from sodium arsenite ($NaAsO_2$, Merck, Germany), disodium hydrogen arsenate ($Na_2HAsO_4 \cdot 7H_2O$) (Wako, Japan), disodium monomethylarsonate ($CH_3AsNa_2O_3 \cdot 6H_2O$) (Chem service, USA) and sodium dimethyl arsinate ($(CH_3)_2AsO_2Na \cdot 3H_2O$) (Merck, Germany), respectively. Standard stock solutions at a concentration of 1000 mg/L (based on As) were prepared by dissolving the respective compounds in water and kept at 4°C. The certified reference materials BCR-627 tuna fish tissue (Brussels, Belgium) and DORM-2 dogfish muscle (National Research Council, Ottawa, Canada) were used to confirm analytical methodologies.

II. *Samples and Sample Preparation*

A total of 60 fish samples were collected from traditional markets and supermarkets located in central Taiwan (i.e. Taichung, Changhwa and Nantou). Table 1 lists the name and number of samples. Fish muscle samples were freeze-dried, ground and homogenized, and sealed in amber glass bottles at 4°C until analysis. The moisture content of samples was determined based on fresh and dried sample weight.

III. *Extraction and Speciation of Arsenic Species from Sample*

0.3 g of sample was extracted with 70 mL of MeOH and H_2O mixed solution (8:2, v/v) using the Soxhlet extraction system (FOSS Sotec™ Avanti 2050, Sweden). The sample was boiled at 120°C for 20 min, then refluxed at 120°C for 30 minutes. Afterwards, the extract was then concentrated to 50 mL and analyzed for arsenic species by high performance liquid chromatography combined with inductively coupled plasma mass spectrometry (HPLC-ICPMS). A Hamilton PRP-X100 anion exchange column (150 × 4.1 mm, trimethylammonium polystyrene divinylbenzene coplymer; Hamilton Company, Reno, NV, USA) was used to separate the arsenic species. Before connecting to HPLC, the ICPMS was tuned for sensitivity, reduced oxides, and doubly charged species using a standard tuning solution (10 ng/g solution of Li, Y, Ce, and Tl in 1% HNO_3). The ion intensity at *m/z* 35 (^{35} Cl) was monitored to correct the interference on *m/z* 75 (^{75}As). Tables 2 and 3 present the instrumental conditions for HPLC and ICPMS, respectively.

Table 1. Fish samples from market

Name	No.
Cephalopod	
Cuttle fishes (*Sepia esculenta Hoyle*)	5
Neritic squid (*Loligo edulis Hoyle*)	5
Octopus (*Octopodidae*)	5
Squids (*Ommastrephidae*)	5
Small fish (body weight ≦ 2 kg/fish)	
Hairtail (*Trichiurus lepturus Linnaeus*)	2
Red sea-bream (*Dentex tumifrons*)	2
Tilapia (*Oreochromis hybrids*)	6
Black sea-bream (*Acanthopagrus schlegeli*)	3
Common sea bass (*Lateolabrax japonicus*)	1
Milkfish (*Chanos chanos*)	2
Large fish (body weight ≧ 10 kg/fish)	
Spanish mackerel (*Scomberomorus commerson*)	2
Marlin (*Makaira indica*)	5
Tuna[a]	4
Salmon[a]	5
Shark[a]	4
Atlantic halibut (*Hippoglossus hippoglossus*)	4

[a] unrecognizable.

IV. *Determination of Total Arsenic*

Total arsenic content of samples was determined after digesting 0.2 g of each sample with 5 mL conc. HNO_3 and 2 mL H_2SO_4, sequentially. The digestion was reacted with an aqueous solution of 1% $NaBH_4$ and 1%

Table 2. Chromatographic conditions used for arsenic speciation analysis

HPLC	Agilent 1050
Column	Hamilton PRP-X100
	Anion-exchange 4.1 mm i.d. × 250 mm, 10 μm
Mobile phase A	0.25 mmol/L $NH_4H_2PO_4$: MeOH (2%, v/v, pH 9.0)
Mobile phase B	20 mmol/L $NH_4H_2PO_4$: MeOH (2%, v/v, pH 8.8)
Gradient program	100% A for 5 min, decreasing to 100% B in 0.1 min and maintaining for 10 min. Regeneration for 10 min with 100% A.

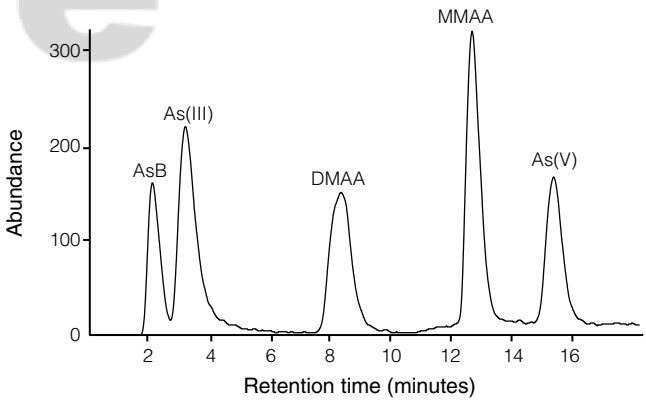

Figure 1. Chromatogram of five different arsenic standards: AsB, As(III), DMAA, MMAA, and As(V), the concentration is 5 μg/L as As.

Table 3. Instrumental conditions of the ICPMS

Parameter	Setting/Type
ICP-MS	Agilent 7500 ce
RF power	1500 W
Carrier gas	1 L/min
Makeup gas	0.15 L/min
Optional gas	Oxygen at 20%
Nebulizer pump	0.15 rps
S/C temp.	2 °C
Monitored ion m/z	75(^{75}As), 35 (^{35} Cl)
Dwell time	410 ms/mass
Total acquisition time	1080 sec

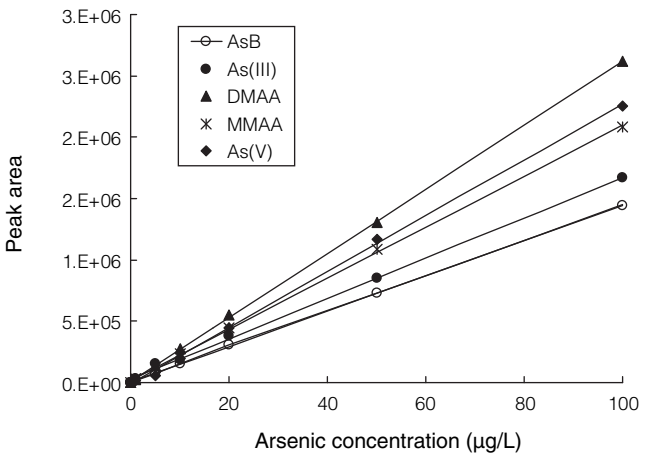

Figure 2. Calibration curves for DMAA, As (V), MMAA, As(III), and AsB over a range of 0 to 100 μg/L as As.

NaOH. The amount of As was determined by a JOBIN-YVON JY-138 ULTRACE ICP-AES equipped with a hydride generator[30].

RESULTS AND DISCUSSION

I. *Determination of As Species in Fish Muscle*

Figure 1 presents the chromatograms for AsB, As(III), DMAA, MMAA, and As(V). The chromatograms on Figure 1 show that arsenic species were separated by gradient anion exchange HPLC using the mobile phase of $NH_4H_2PO_4$ and MeOH mixed solution. The separation was completed in approximately 20 min. Figure 2 shows the linearity (r^2 = 0.9984 - 0.9999) of calibration curves for different arsenic species over a range of 0 - 100 μg/L as As. The recovery rates of AsB, As(III), DMAA, MMAA, and As(V) by spiking 0.133 mg/kg As to the muscle of swordfish are 95%, 85%, 111%, 83% and 84%, respectively. Table 4 presents analytical results for standard reference materials. Recovery rates of analysis of AsB in BCR 627 (tuna fish) and DORM-2 (dogfish muscle) were 95% and 90%, respectively. To analyze DMAA in BCR, the recovery rate was 113%. The recovery rates for total As determination of BCR 627 and DORM-2 were 108% and 101%, respectively. The detec-

Table 4. Analysis results of standard reference materials (mg/kg As)

SRM (No.)	AsB	DMAA	Total As
Tuna fish (BCR 627)	3.90 ± 0.22[a] (3.72 ± 0.10)[b]	0.15 ± 0.02 (0.17 ± 0.02)	4.8 ± 0.3 (5.20 ± 0.10)
Dogfish muscle (DORM-2)	16.4 ± 1.1 (14.8 ± 0.3)	--	18.0 ± 1.1 (18.1 ± 0.2)

[a] Certified value.
[b] Value detected, data are means ± standard deviations of three replications.

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tion limits were calculated based on 3 δ of baseline noise at the retention time of peak (n = 7). The detection limits for AsB, As(III), DMAA, MMAA, and As(V) were 0.02, 0.01, 0.02, 0.01 and 0.02 mg/kg as As, respectively.

II. *Arsenic Species Content in Fish Muscle Samples*

Table 5 lists the content of total As and arsenic species in fish muscles. The DMAA, MMAA, and As(III) contents in all samples were undetectable. Comparing average total As (TAs) content in different samples demonstrated that the cephalopods have higher TAs content than small and large fish. The comparison also indicated lack of difference between TAs content in small and large fish samples (Table 5). The TAs content in cephalopods, small fish and large fish were 5.91, 1.93 and 1.96 mg/kg, respectively. According to the report by the GESAMP (IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP joint group experts on the scientific aspects of marine pollution)[31], the As content in most commercial fish species was 1 mg/kg. Fish living in benthic habits and mollusks have arsenic concentrations near 10 mg/kg. Specifically, cephalopods have occasionally been reported to contain relatively higher arsenic concentrations. The As content in marine fish species from different areas was 0.12 - 52 mg/kg[32]. Arsenic can be magnified in the aquatic food chain[6]. High values may reflect environmental pollution. Freshwater fish in Africa contain 0.004 - 0.38 mg/kg As[33]. The Food Standards Agency of the UK, which completed a survey of TAs and inorganic As in edible portions of 42 composite fish samples, concluded that average TAs and inorganic As contents were 3.39 and 0.02 mg/kg, respectively, and total As content was 0.12 - 20.2 mg/kg[34]. In this study, the TAs content ranged between 0.39 and 23.0 mg/kg (Table 5).

The inorganic As species of As(V) contents in cephalopods, small and large fish samples were 0.10, 0.09 and 0.10 mg/kg As (fresh weight), respectively (Table 5). No significant difference for As(V) content existed between the three sample types. However, the ratios of As(V) to TAs in cephalopods, small and large fish samples were 1.7, 4.7 and 5.1%, respectively. Average AsB content in cephalopods, small and large fish were 5.42, 1.57 and 1.54 mg/kg As, respectively (Table 5). AsB concentrations were 0.31 - 20.1 mg/kg As in all samples. Therefore, AsB content in cephalopods was higher than that in small and large fish. No significant difference existed for AsB content between small and large fish. Arsenic, mainly in organic forms, occurs in marine organisms used as human food (fish, crustaceans, mollusks, and algae). Organic arsenic is present in almost all marine animal species, chiefly as AsB[14]. Moreover, AsB is the major or sole arsenical in both teleost and elasmobranch fish[31]. Arsenobetaine was detected as the major As compound in 61 seafood samples collected from the Aegean Sea in Greece; the concentration was 2.7 - 23.1 μg/g (dry weight). The ratio of AsB to TAs was greater than 90%, whereas inorganic As compounds were undetectable. The DMAA was detected in only two samples[35]. The percentage of AsB to TAs in samples in this study was 54 - 100%. Average percentages of AsB to TAs in cephalopods, small and large fish were 87, 82 and 79%, respectively (Table 5). These results indicated that AsB is the predominant arsenic compound in fish samples.

III. *Evaluation of Dietary Intake of As from Fish in Taiwan*

Based on the As content in fish muscle samples (Table 5), average TAs and AsB contents in fish samples were 3.27 and 2.84 mg/kg As, respectively. Weekly intake of fishery products by Taiwanese residents is 561.4 g/person/week[36]. Weekly dietary intake of As from eating fish was determined based on the average TAs and AsB content in fish muscle samples and weekly intake of fishery products. Thus, the weekly intake of TAs and AsB was 30.6 and 26.6 μg/kg body weight/week, respectively (Table 6). Although the weekly intake of TAs (30.6 μg/kg body weight/week) is higher than the provincial tolerable weekly intake of 15 μg/kg body weight/week for inorganic arsenic recommended by the WHO[18],

Table 5. Content of total As and arsenic species in fish muscle samples (mg/kg As, on fresh weight basis)[a]

Sample (No.)	Total As	As(V)	AsB	AsB/Total As (%)
Cephalopod	5.91 ± 7.80b[b]	0.10 ± 0.05[a]	5.42 ± 7.24[b]	87
(20)	(0.39-23.0)[c]	(0.02-0.19)	(0.31-20.1)	(69-100)
Small shape fish	1.93 ± 1.98[a]	0.09 ± 0.05[a]	1.57 ± 1.62[a]	82
(16)	(0.71-7.44)	(0.03-0.18)	(0.58-6.05)	(63-97)
Large shape fish	1.96 ± 1.20[a]	0.10 ± 0.06[a]	1.54 ± 1.13[a]	79
(24)	(0.74-6.06)	(0.03-0.34)	(0.49-6.03)	(54-100)

[a] DMAA, MMAA and As(III) were undetectable.
[b] Data are mean ± standard deviations. Values in the same column followed by letters are significantly different ($p < 0.05$) by Duncan's multiple range test.
[c] Range.

Table 6. Weekly intake of As from eating fish (μg/kg body weight/week)

	Total As	AsB
Weekly intake[a]	30.6	26.6
Provincial tolerable weekly intake[b] (inorganic As)	15	--

[a] The weekly intake of fishery product is 561.4 g/person/week, the average body weight is 60 kg/person.
[b] WHO, 1989.

it contained 87% AsB (26.6 μg/kg body weight/week). Notably, AsB is considered nontoxic and non-carcinogenic to mammals. Furthermore, the half-life of AsB in humans is short (6 hours), and is excreted easily after intake by mammals[12-16]. Therefore, based on experimental results, the risk of As intake by eating fish is low in Taiwan.

CONCLUSIONS

The arsenic species including AsB, DMAA, MMAA, arsenate and arsenite in fish muscle were extracted using a methanol and water mixture, then determined by HPLC-ICPMS. The analytical results of a total of 60 fish samples indicated that DMAA, MMAA and As(III) content was undetectable in all samples. The As(V) content in all samples ranged from 0.03 to 0.34 mg/kg As. No difference existed between TAs content of small and large fish samples. Cephalopods had higher TAs and AsB contents than small and large fish. Results also revealed that AsB is the main As compound found in fish muscle. Percentages of AsB to TAs in samples were 54–100%. Weekly intake of As by Taiwanese residents was 30.6 μg/kg body weight/week calculated based on fish consumption. Although it is higher than the acceptable weekly intake of 15 μg/kg body weight/week (inorganic As) suggested by the WHO, 87% of the As in fishery products was the nontoxic compound AsB. Therefore, the risk of As poisoning due to fish muscle intake is low in Taiwan.

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REFERENCES

1. Shamberger, R. J. 1979. Beneficial effects of trace elements. In "Toxicity of Heavy Metals in the Environment". pp. 689-796. Oehme, F. W. ed. Marcel Dekker, New York, U. S. A.
2. Kabata-Pendias, A. and Pendias, H. 1986. Trace elements in soil and plants. CRC Press, Boca Raton, FL. U. S. A.
3. Farmer, J. G., Johnson, L. R. and Lovell, M. A. 1989. Urinary arsenic speciation and the assessment of UK dietary, environmental and occupational exposures to arsenic. Environ. Geochem. Health. 11: 93-95.
4. Korte, N. E. and Fernando, Q. 1991. A review of arsenic (III) in groundwater. Crit. Rev. Environ. Control. 21: 1-39.
5. Tseng, W. P. 1977. Effects and dose-responds relationships of skin cancer and black foot disease with arsenic. Environ. Health Persp. 19: 109-119.
6. WHO. 1989. Arsenic. Environmental Health Criteria. No. 18. pp. 174 Geneva, Switzerland.
7. Ishinishi, N., Tsuchiya, K., Vahter, M. and Fowler, B. A. 1986. Arsenic. In "Handbook on the Toxicology of Metals". 2nd ed. pp. 43-83. Friberg, L., Nordberg, G. F. and Vouk, V. B. ed. Elsevier Sci. Publ. New York, U. S. A.
8. International Agency for Research on Cancer. 1987. Arsenic and arsenic compounds (Group 1). IARC Monograph 23, Supplement 7. pp. 100-106. Lyon, France.
9. Fisherbein, L. 1984. Overview of analysis of carcinogenic and/or mutagenic metals in biological and environmental samples. I. Arsenic, beryllium, cadmium, chromium, and selenium. J. Environ. Anal. Chem. 17: 113-170.
10. Bhumbla, D. K. and Keefer, R. F. 1994. Arsenic mobilization and bioavailability in soils. In "Arsenic in the Environment. Part 1: Cycling and Characterization". pp. 51-82. Nriagu, J. O. ed. John Wiley and Sons. New York, U. S. A.
11. Leermakers, M., Baeyens, W., De Gieter, M., Smedits, B., Meert, C., De Bisschop, H. C., Morabito, R. and Quevauviller, Ph. 2006. Toxic arsenic compounds in environmental samples: Speciation and validation. Trends Analyt. Chem. 25: 1-10.
12. Brisbin, J. A., Hymer, C. B. and Caruso, J. A. 2002. A gradient anion exchange chromatographic method for the speciation of arsenic in lobster tissue extracts. Talanta. 58: 133-145.
13. Neff, J. M. 1997. Ecotoxicology of arsenic in the marine environment. Environ. Toxic. and Chem.16: 917-927.
14. Edmonds, J. S. and Francesconi, K. A. 1993. Arsenic in seafoods: Human health aspects and regulations. Marine Poll. Bull. 26: 665-674.
15. Yamauchi, H. and Yamamura, Y. 1984. Metabolism and excretion of orally ingested trimethylarsenic in man. Bull. Environ. Contam. Toxicol. 32: 682-687.
16. Luten, J. B., Riekwel-Booy, G. and Rauchbaar, A. 1982. Occurrence of arsenic in plaice (*Pleuronectes platessa*), nature of organo-arsenic compound present and its excretion by man. Environ. Health Perspect. 45:

165-170.
17. Lin, H. T. 2004. Heavy metal content in the meat of fish and livestock. Project report of Council of Agriculture (93AS-8.2.3-P-P1) (un-publish).
18. WHO. 1988. Arsenic. WHO Food Additives Series. 24 http://www.inchem.org/documents/jecfa/jecmono/v024je08.htm
19. Pizarro, I., Gómez, M., Cámara, C. and Palacios, M. A. 2003. Arsenic speciation in environmental and biological samples extraction and stability studies. Analytica Chimica Acta. 495: 85-98.
20. Falk, K. and Emons, H. 2000. Speciation of arsenic compounds by ion-exchange HPLC-ICP-MS with different nebulizers. J. Anal. At. Spectrom. 15: 643-649.
21. Hirata, S., Toshimitsu, H. and Aihara, M. 2006. Determination of arsenic species in marine samples. Anal. Sci. 22: 39-43.
22. Ackley, K. L., Hymer, C. B., Sutton, K. L. and Caruso, J. A. 1999. Speciation of arsenic in fish tissue using microwave-assisted extraction followed by HPLC-ICP-MS. J. Anal. At. Spectrom 14: 845-850.
23. Gong, Z., Lu, X., Ma, M., Watt, C. and Le, X. C. 2002. Arsenic speciation analysis. Talanta. 58: 77-96.
24. Nam, S. H., Cheng, J., Mindak, W. R. and Capar, S. G. 2006. Preliminary results of extraction, separation and quantitation of arsenic species in food and dietary supplements by HPLC-ICP-MS. Bull. Korean Chem. Soc. 27: 903-908.
25. Morita, M. and Edmonds, J. S. 1992. Determination of arsenic species in environmental and biological samples. Pure & Appl. Chem. 64: 575-590.
26. Le, X. C. 1999. Arsenic speciation in the environment. ACCN (Canadian Chemical News). September: 18-20.
27. Le, X. C., Lu, X. and Li, X. -F. 2004. Arsenic speciation. J. Anal. Chem. 1: 27A-33A.
28. Wahlen, R. 2004. Fast and accurate determination of arsenobetaine in fish tissue using accelerated solvent extraction and HPLC-ICP-MS determination. J. Chrom. Sci. 42: 217-222.
29. PerkinElmersciex. 2006. Speciation of five arsenic compounds in urine by HPLC/ICP-MS. http://www.perkinelmer.com
30. Lin, H. T., Wong, S. S. and Li, G. C. 2004. Heavy metal content of rice and shellfish in Taiwan. J. Food Drug Anal. 12: 167-174.
31. GESAMP. 1986. Review of potentially harmful substances: arsenic, mercury and selenium. GESAMP Reports and Studies. 28.
32. Madany, I. M., Wahab, A. A. A. and Al-Alawi, Z. 1995. Trace metals concentrations in marine organisms from the coastal areas of Bahrain gulf. Water Air Soil Poll. 91: 233-248.
33. Biney, C., Amuzu, A. T., Calamari, D., Kaba, N., Mbome, I. L., Naeve, H., Ochumba, P. B. O., Osibanjo, O., Radegonde, V. and Saad, M. A. H. 1994. Review of heavy metals in the African aquatic environment. Ecotoxicol. Environ. Saf. 28: 134-159.
34. Food Standards Agency. 2005. Survey of arsenic in fish and shellfish. http://www.food.gov.uk/science/surveillance/fsis2005/fsis8205
35. Schaeffer, S. R., Ipolyi, C. I., Fodor, P. and Thmaidis, N. S. 2005. Determination of arsenic in seafood samples from the Aegean sea by liquid chromatography-(photo-oxidation)-hydride generation-atomic fluorescence spectrometry. Anal. Chim. Acta 547: 109-118.
36. Pesticide Residue Division, Taiwan Agricultural Chemicals and Toxic Substances Research Institutes. 2001. The estimation of daily intake by Taiwanese (in Chinese) (un-publish).