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Speciation Analysis of Arsenic in Food Samples with High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry

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Abstract: The speciation of arsenic in food samples was analyzed. The methods used for sample preparation include: the extraction of arsenic from food samples using a 1∶1 (v/v) methanol/water, and high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) determination of the speciation of arsenic in food samples. A C18 column was used to separate the arsenic species. The mobile phase consists of 5 mmol/L tetrabutylammonium hydride, 2 mmol/L malonic acid and 5% (v/v) methanol solution with the pH adjusted to 5.9. The mobile phase flow rate was 1.2 mL/min. The chemical species of arsenic present in the foodstuffs were found mainly As (Ⅲ), As (Ⅴ), dimethylarsinic (Ⅴ) (DMA (Ⅴ)). Detection limit was based on signal to noise ratio of 3. As to apple samples, the detection limits were 0.2, 0.6 and 0.3 μg/kg for As (Ⅲ), As (Ⅴ) and DMA (Ⅴ), respectively. The concentrations of arsenic in raw apple samples were calculated.

Key words: high performance liquid chromatography; mass spectrometry; organic arsenic; inorganic arsenic; food
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高效液相色谱-等离子质谱联用分析食品中的主要有机砷和无机砷

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摘要: 应用液相色谱-等离子质谱联用的方法分析食品样品中的主要有机砷(一甲基砷和二甲基砷)和无机砷(三价砷和五价砷)。采用 50%(体积分数)甲醇水溶液作为萃取剂,将食品样品进行预处理,再以 5 mmol/L四丁氢铵,2 mmol/L丙二酸和 5%(体积分数)甲醇水溶液作为流动相(pH 5.9),C18 色谱柱(150 mm×4 mm i.d., 5 μm)将样品萃取液进行液相色谱分离,最后进入等离子质谱仪定性分析。经测定发现,新鲜蔬菜和水果样品中主要含有的无机砷为三价砷和五价砷,有机砷为二甲基砷。一甲基砷在个别样品中也有发现。该法的检出限分别为:三价砷 0.2 μg/kg,五价砷 0.6 μg/kg和二甲基砷 0.3 μg/kg。

关键词: 高效液相色谱; 质谱; 有机砷; 无机砷; 食品

Arsenic, a human carcinogen to cause skin, lung and bladder cancer following chronic exposure, has long been recognized for its toxicity[1]. It is a common practice to express arsenic exposure in terms of elemental arsenic (As) but this masks the pharmacokinetic and toxicological differences of the range of arsenic compounds present in the environment.

Arsenic is rarely present in free state in the environment but is widely distributed as both inorganic and organic compounds. With the development of analytical techniques, much research on the identification of arsenic species has been done so far. It has shown that the toxicity of arsenic is highly dependent on its chemical form[2]. Different forms of arsenic possess different toxicological properties. In general, inorganic arsenic (arsenite and arsenate) is more toxic than monomethylarsonic (MMA), dimethylarsinic (DMA) and other organic arsenics. Groups of arsenical compounds have been ranked in decreasing order of toxicity: As(Ⅴ) > As(Ⅲ) > MMA(Ⅴ) > DMA(Ⅴ) > Arsenosugars)[3]. Therefore, total arsenic concentration is not an adequate measure for estimating risk from arsenic present in food and drinking water supplies[2,4,5].

Arsenic has been found in foodstuffs while it also exists in air, water and soil, but the crucial question is at what concentration and at what speciation. With the exceptions of seafoods, and animal and poultry of-

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fal, the concentration of arsenic in food appears to be generally <0.25 mg/kg. Unfortunately, because the methods of extraction from foodstuffs may not be efficient enough or may destroy the original species present, little is known about the arsenic species in most foods people eat now[3]. In addition, identification of the arsenic chemical species and contents in foodstuffs with endemic arsenicism would assist in the assessment of tolerable arsenic intakes by ingestion of food and water. Thus, more attention needs to be given to the speciation of arsenic species in foods.

The method used to analyze arsenic in prepared samples has been extensively studied previously. In this case we used 1∶1 (v/v) methanol/water, which was reported to be efficient enough[2]. Then high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) was used to analyze the speciation of arsenic in the extracts of food samples. The chemical species of arsenic present in the foodstuffs were found mainly As(Ⅲ), As(Ⅴ), DMA(Ⅴ) and MMA(Ⅴ)[2].

1 Experimental

1.1 Apparatus

A Perkin Elmer Series 200 Pump, a Peltier Column Oven and an Autosampler (PE, USA) were used for the separation of arsenic species. Sample injection volumes were 20 μL. A C18 column (Phenomenex, USA, 150 mm×4 mm i.d., 5 μm) was used to separate the arsenic species. The mobile phase consists of 5 mmol/L tetrabutylammonium hydride, 2 mmol/L malonic acid and 5% (v/v) methanol solution with pH adjusted to 5.9. The mobile phase flow rate was 1.2 mL/min[6]. ICP-MS was used to analyze total arsenic in food samples. And ICP-MS coupled with HPLC was used to determine a complete speciation on arsenic samples. The ICP-MS instrument was an ELAN DRC Plus 6100 (Perkin Elmer, Sciex, USA). The ICP-MS operating parameters were as follows: nebulizer gas flow rate 0.87 L/min, auxiliary Ar flow rate 1.20 L/min, plasma gas flow rate 15.00 L/min, lens voltage 1 100.00 V, analog stage voltage −2 000.00 V, and pulse stage voltage 1 250.00 V. The ICP-MS required an injection volume of approximately 20 L. ICP-MS tuning was performed daily with multi-element solutions.

1.2 Reagents and chemicals

Sodium arsenate $As(O)OH(ONa)_2 \cdot 7H_2O$ and sodium cacodylate $(CH_3)_2As(O)ONa$ were obtained from Sigma (St. Louis, MO, USA). Stock solutions (1 000 mg/L) were prepared by dissolving arsenic compounds in 0.01 mmol/L hydrocholoric acid, and standard solutions were prepared by serial dilution with deionized water.

An atomic absorption arsenic standard solution (Sigma) containing 1 000.00 mg/L arsenic in 20 g/L KOH was used as the primary arsenic standard. Concentrations of arsenic in sodium arsenate, sodium cacodylate, and sodium monomethylarsinate solutions were standardized against the atomic absorption arsenic standard solution using both ICP-MS and flame atomic absorption spectrometry analysis. The source of MMA (Ⅲ) was solid oxide (CH_3AsO), which was prepared following the procedure of Cullen *et al*. Tetrabutylammonium hydroxide as an ion-pairing reagent and malonic acid as a buffer for HPLC separation were from Aldrich (Milwaukee, WI, USA). HPLC grade methanol was from Fisher (Pittsburgh, PA, USA). The mobile phase solutions (pH 5.9) containing 5 mmol/L tetrabutylammonium hydroxide, 2 − 5 mmol/L malonic acid, and 5% methanol were prepared in deionized water and filtered through a 0.2 μm membrane before use. Sodium borohydride (Fisher) was freshly prepared daily. All reagents used were of analytical grade or better. All the food samples were purchased from Safeway and Saveon Food (a superstore of Canada). The samples were ground and blended before use.

1.3 Sample preparation

About 2 g ground sample was weighed and placed in a 50 mL plastic centrifuge tube. Twenty milliliter 1∶1 methanol/water solution was added to the tube. After being sonicated for 30 min, the sample was centrifuged at 3 000 r/min for 5 min, and then filtered. The first extract was collected and the residue was added with 20 mL 1∶1 methanol/water solution and sonicated for 30 min, then centrifuged for 5 min at 3 000 r/min, filtered again and the second extract of the residue was collected and mixed with the first extract. All the extracts were evaporated at 40 ℃ until about 1 mL left. The sample was diluted to 2 mL for the analysis of arsenic speciation by HPLC-ICP-MS. Blank samples were identically prepared to verify that no arsenic was introduced from an

outside source.

2 Results

For speciation work, rice, fresh vegetable, some raw fruit and two kinds of herb cablet samples were extracted using the sonication-centrifugation procedure with 1∶1 methanol/water. The extracts were analyzed by HPLC-ICP-MS. Fig. 1 shows the separation obtained for a standard containing four species at a concentration of 10 μg/kg for each component. As(Ⅲ), DMA(Ⅴ), MMA(Ⅴ) and As(Ⅴ) were baseline resolved within 7 min. The speciation of arsenic results are shown in Table 1 and an example chromatogram for ginger sample was shown in Fig. 2. The quantity of arsenic speciation in raw apple samples results were shown in Table 2. Inorganic arsenic was found almost in all the food samples analyzed. The organic arsenic was mostly DMA (Ⅴ) though MMA(Ⅴ) was detected in an apple sample and ginseng caplets. Sensitivity of ICP-MS instrument changed from day to day. Detection limits were based on signal to noise ratio of 3. As to apple samples, the detection limits are 0.2, 0.6 and 0.3 μg/kg for As (Ⅲ), As(Ⅴ) and DMA(Ⅴ), respectively.

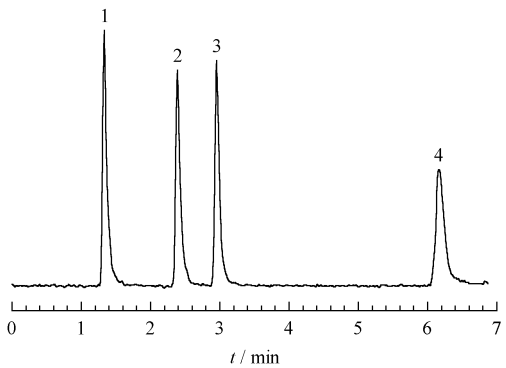

Fig. 1 Chromatogram of a standard mixture containing four arsenic species

Peaks：1. As(Ⅲ)；2. DMA(Ⅴ)；3. MMA(Ⅴ)；4. As(Ⅴ).

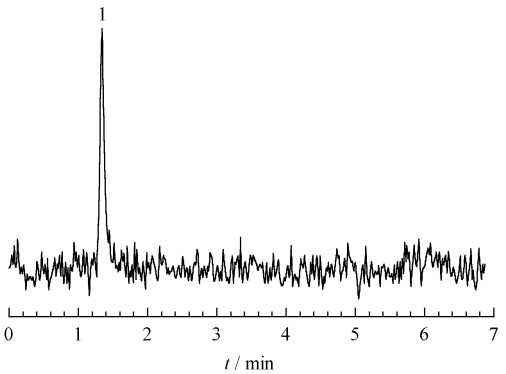

Fig. 2 Chromatogram of a ginger sample

Peak：1. As (Ⅲ).

Table 1 Arsenic species in food samples μg/kg

Food sample	As(Ⅲ)	As(Ⅴ)	MMA(Ⅴ)	DMA(Ⅴ)	Unidentified arsenic
Rice Krispies cereal (Kellogg 's, Canada)	0.9	n.d.	n.d.	1.2	RT：4.1 min
Special K cereal (Kellogg 's, Canada)	0.8	n.d.	n.d.	0.4	RT：4.1 min
Rice(Kokuho Rose, USA)	1.7	1.2	n.d.	0.9	n.d.
Rice(Diamond G, USA)	1.1	1.1	n.d.	0.4	n.d.
Rice (Thai Long Grain Rice, Thailand)	1.3	1.3	n.d.	1.0	n.d.
Rice (Dagongji, Thailand)	1.4	2.4	n.d.	1.3	RT_1：2.7 min RT_2：6.7 min
Rice(Jesmine Fragrant Rice, Thailand)	1.3	1.3	n.d.	0.8	RT_1：2.7 min RT_2：6.7 min
Red pepper	n.d.	n.d.	n.d.	n.d.	n.d.
Green pepper	5.8	8.9	n.d.	n.d.	n.d.
Mushroom	2.7	n.d.	n.d.	2.6	n.d.
Carrot	5.7	9.1	n.d.	n.d.	n.d.
Spinach	0.9	1.4	n.d.	n.d.	RT_1：1.1 min RT_2：5.0 min
Broccoli	0.6	0.6	n.d.	n.d.	RT_1：1.1 min RT_2：5.0 min
Celery	1.2	n.d.	n.d.	n.d.	RT_1：1.1 min RT_2：4.5 min
Romaine	2.4	1.3	n.d.	n.d.	n.d.
Ginger	0.8	n.d.	n.d.	n.d.	n.d.
Orange	n.d.	0.6	n.d.	n.d.	n.d.
Kiwi(Zespri Green,New Zealand)	n.d.	0.5	n.d.	0.3	n.d.
Ginkgo Biloba (Life, Canada)	20.2	26.8	n.d.	9.9	n.d.
Ginseng (Life, Canada)	46.5	61.4	258.3	9.2	n.d.

RT：retention time. n.d.：not detected.

Table 2 Arsenic speciation analysis in raw apple samples μg/kg

Sample No.	As(Ⅲ)	DMA(Ⅴ)	MMA(Ⅴ)	As(Ⅴ)
1	n.d.	n.d.	n.d.	n.d.
2	0.2	0.3	n.d.	n.d.
3	n.d.	n.d.	n.d.	n.d.
4	0.4	0.4	n.d.	n.d.
5	5.6	2.5	n.d.	0.8
6	0.3	0.6	n.d.	n.d.

3 Discussions

HPLC-ICP-MS provides a sensitive and highly selective method for the identification of arsenic speciation in food samples. As(Ⅲ), As(Ⅴ) and DMA(Ⅴ) were the predominant arsenic species detected in most food samples analyzed. MMA(Ⅴ) was also detected in a few food samples analyzed. Extraction procedures for removing arsenic species from raw apple samples were evaluated. The method used to extract the food samples with 1∶1 methanol/water is efficient enough for the identification of arsenic speciation of food samples by HPLC-ICP-MS. There is a need to develop a simple and more efficient method for the extraction of food samples so that it will meet the high demands of analysis of arsenic speciation in food samples.

Based on the results of arsenic of the same kind of raw apple samples but from different areas, the following general conclusions can be drawn. The speciation of arsenic of the same kind of food might be different from area to area, so does the toxicity of arsenic in the foods from different areas. The reasons might be that different pesticides or different concentrations are applied and the soil properties are different.

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