http://dx.doi.org/10.5012/bkcs.2013.34.11.3206

An Investigation on Inorganic Arsenic in Seaweed by Ion Chromatography Combined with Inductively Coupled Plasma-Atomic Emission Spectrometry

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Received July 14, 2013, Accepted August 1, 2013

This study focused on the matrix interference for the qualitative and quantitative analysis of inorganic arsenic species in seaweed by ion chromatography (IC) combined with inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The matrix of seaweed has a significant effect on the determination of inorganic arsenic species. In particular, the retention times of inorganic arsenic species in the in the standard solution were different from those in seaweed because of the matrix interference. Thus, it was not suitable to use the chromatographic method for the determination of the arsenic species in seaweed. We investigated an alternative method for the determination of inorganic arsenic species in seaweed. The method was applied for the seaweed samples such as laver, green laver, sea tangle and sea mustard. The sample extraction methods of the arsenic species were also investigated in this study.

Key Words : Inorganic arsenic species, Seaweed, Matrix interference, ICP-AES

Introduction

Because inorganic arsenic is a highly toxic substance and exist in our environment universally, the determination of arsenic has been very important.[1,2] Symptoms of arsenic poisoning begin with headaches, confusion and drowsiness. The final result of arsenic poisoning is coma or death.[3,4] The most arsenic compounds dissolve in water. Thus, arsenic can be found in various kinds of foods. in environment, arsenic mostly exists as As^{3+} and As^{5+} forms that has a carcinogenic effect.[5,6] The route of human ingestion of arsenic is mainly through foods, especially seafoods containing high level of arsenic.[7] The bioaccumulation of arsenic in seafood has been reported as 0.5-50 mg/kg.[8,9] The Food and Agriculture Organization/World Health Organization (FAO/WHO) suggests a provisional tolerable weekly intake (PTWI) of 15 μg inorganic arsenic per 1 kg body weight[10] and recommends that the arsenic level of drinking water and environmental water should not be more than 10 μg/L.[11]

The toxicity and biological effects of arsenic depend on its chemical species.[12] Therefore, both total arsenic and each individual arsenic species should be determined in various samples.[13,14] Many studies has been devoted to the analysis of the arsenic in various samples so far. Geng *et al.* determined the arsenic species in marine product samples using solvent extraction-high performance liquid chromatography (HPLC) and digestion-cryogenic trap method.[15] Al-Assaf *et al.* determined four arsenic species in soil by sequential extraction and HPLC with post-column hydride generation and inductively coupled plasma-atomic emission spectrometry (ICP-AES) detection.[16] Richard Schaeffer *et al.* determined the arsenic species in seafood by liquid chromatography (photo-oxidation) hydride generation atomic fluorescence spectrometry.[17] Pasias *et al.* determined the total arsenic and total inorganic arsenic and inorganic arsenic species in rice and rice flour by electrothermal atomic absorption spectrometry.[18] And Leufroy *et al.* determined seven arsenic species in seafood by ion exchange chromatography coupled to inductively coupled plasma-mass spectrometry following microwave assisted extraction.[19]

It is difficult to determine the arsenic species in samples because the oxidation states of arsenic species changed as a result of the matrix interference of the samples during the process of sample preparation.[20-22] In this study, we learned that the matrix interference gave a significant impact on the analysis of the arsenic species in seaweed. The retention times of the arsenic species in the seaweed were different from those in standard solutions when the arsenic chemical species were determined by IC-ICP-AES. Thus, it was difficult to use the chromatographic method for the determination of the arsenic species in seaweed. An alternative method was used to determine the total inorganic arsenic in seaweed.

Experimental

Instrumentation. An ICP-AES (ARCOS, SPECTRO, Germany) was used for the detection of the concentration of total arsenic. Operating condition is shown as Table 1. For the separation of the species in samples, a GP-50 ion chromatography (DIONEX, USA) was used. Instrument setting is summarized in Table 2. A shaker (VS-202D, VISION, Korea), microwave digestion Q4000 (TEKTON, Canada), ultrasonic extractor (POWER SONIC 420, HWASHIN TECH, Korea), centrifuge (SUPRA21K, HANIL SCIENCE INDUSTRIAL, Korea) and rotary evaporator (VV2008, Heidolph, Germany) were used for sample preparation.

Reagents. Arsenic standard stock solutions for the determination of total arsenic and arsenic species were prepared by dissolving the following reagents in water and stored at 5 °C: Sodium metaarsenite, $NaAsO_2$ (≥ 90%, Sigma-Aldrich,

Table 1. Operating conditions of ICP-AES (SPECTRO, Germany)

Power	1400 W
Pump speed	30 rpm
Argon gas flow rate	
Coolant flow	12 L/min
Auxiliary flow	1.0 L/min
Nebulizer flow	0.8 L/min
Run time	500 s
Sampling rate	2.0 Hz
Integration interval	500 ms
Nebulizer	Cross flow
Monitor Wavelength	189.042 nm
Maximum	24,000,000 cps

Table 2. Operating conditions of IC (DIONEX, USA)

Guard column	IonPac AG7 (50 mm × 4 mm)
Analytical column	IonPac AS7 (250 mm × 4 mm)
Flow rate	1.0 mL/min
Sample loop	100 μL
Mobile phase A	0.5 mM Nitric acid, 0.05 mM Benzene-1,2-disulfonic acid dipotassium salt
Mobile phase B	50 mM Nitric acid, 0.05 mM Benzene-1,2-disulfonic acid dipotassium salt

St. Louis, MO£¨USA), sodium arsenate, $Na_2HAsO_4{\cdot}7H_2O$ (≥ 98%, Sigma-Aldrich), disodium methylarsenate (MMA), $Na_2CH_3AsO_3{\cdot}6H_2O$ (97.5%, Sigma-Aldrich), Dimethylarsinic acid (DMA), $C_2H_7AsO_2$ (98%, Sigma-Aldrich). Ultrapure water (18 MΩ/cm) from a water purification system (PURELAB pRO 20 + PURELAB Plus UF, ELGA, USA) was used for the preparation and dilution of the stock solutions, eluents and samples. Pure nitric acid (70%, Sigma-Aldrich) was used to extract total arsenic. Methanol (99.9%) used to extract arsenic species was purchased from Sigma-Aldrich. Benzene-1,2-disulfonic acid dipotassium salt was purchased from Sigma-Aldrich. Hydrochloric acid (37%), chloroform (≥ 99.8%), hydrobromic acid (48%) and hydrazine sulphate (≥ 99%) were used to determine the inorganic arsenic were purchased from Sigma-Aldrich.

Sample Preparation. The wet seaweed samples were placed on the clean plastic film and dried at room temperature by sunlight. After being dried, those were pulverized to powder using liquid nitrogen. The samples powder were then kept in the refrigerator hermetically.

Results and Discussion

Determination of the Total Arsenic in Seaweed. First of all, an arsenic solution (209 μg/kg arsenic in 1,000 mg/kg Na^+ solution) was prepared to investigate the interference of Na^+ on the determination of arsenic because the seaweed contains lots of sodium chloride.[23] The calibration curve for the determination of total arsenic was obtained using five arsenic standard solutions (0.02, 0.05, 0.1, 0.2 and 0.5 mg/kg arsenic in distilled deionized water). The concentration of the total arsenic in the solution was determined by ICP-AES.

Table 3. Results for arsenic in 1000 mg/kg Na^+ solution[a] (μg/kg)

	Theoretical Value	Measured Value
Concentration of As	209	204 3.4

[a]sample was determined for 3 times

Table 4. Results for the concentrations of total arsenic in seaweed

	Concentration of total arsenic (mg/kg)
Sea Mustard	52.1 ± 0.1
Laver	15.6 ± 0.5
Green Laver	3.62 ± 0.15
Sea Tangle	52.0 ± 0.1

The results were shown in the Table 3. It showed no significant interference of Na^+ on the determination of arsenic. The theoretical value was 209 μg/kg, and the measured value was 204 ± 3.4 μg/kg (200.6-207.4 μg/kg).

A dried seaweed sample (0.2000 g) was transferred to a 30 mL polypropylene centrifuge tube. Then 10 mL of the extraction solvent (10% HNO_3) was added to the tube and the sample was shaken at room temperature for 24 h. After the shaking process, a centrifuge was used to separate the sample from the extraction solvent. The solution was filtered through a 0.2 μm filter and the final volume was made to 30 mL. Then, the total arsenic in the sample was determined by ICP-AES. The results were shown in Table 4. The concentrations of the total arsenic in the sea mustard and sea tangle were found to be much higher than those in the laver and green laver.

Extraction of Arsenic Species using HNO_3. The extraction of different arsenic species from the various samples is still complicated and difficult because the integrity of the different species should not be changed during the sample preparation.[24] In this study, HNO_3 was investigated for the extraction solvent because HNO_3 has been generally used for the extraction.[25,26] A standard solution of 0.5 mg/kg arsenic species (As^{3+}, As^{5+}, MMA and DMA) was prepared by using 3% and 10% HNO_3 as solvents to make sure if the

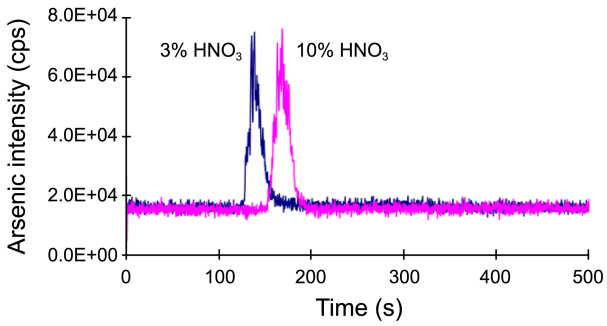

Figure 1. Chromatogram for arsenic species in 3% and 10% HNO_3 solution.

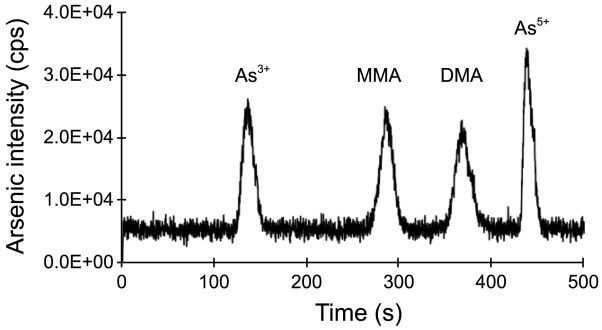

Figure 2. Chromatogram for arsenic species in aqueous solution.

different chemical species of arsenic changed in the process of extraction. The solution was injected into IC coupled with the ICP-AES, and the chromatograms were shown in Figure 1. The same solution was also made in the aqueous solution to compare the above chromatogram (Figure 2).

From the chromatograms we can find that the arsenic in an aqueous solution could be fully separated while there was only one peak obtained by different arsenics in HNO_3 solution. The arsenic species in HNO_3 solutions could not be separated. The reasons might be due to the change of the retention times and/or the oxidation states of the arsenic species by the interference of HNO_3. This result indicates that HNO_3 was not suitable for the extraction of the arsenic species from the seaweed.

Extraction of Arsenic Species using MeOH. MeOH was also investigated for the extraction solvent.[27,28] A multi solution (0.5 mg/kg As^{3+}, As^{5+}, MMA and DMA) was prepared with solvents of 5%, 10%, 20% and 50% MeOH in water (v/v), respectively. The results were shown in Figure 3. MeOH did not affect the retention time in the column, but the signal intensities of As^{3+} were enhanced with the MeOH. The enhancement of As^{3+} might be due to the increase of electron number density by carbons from MeOH in the plasma.

The results showed the MeOH should be removed after the extraction of arsenic species from the sample. Thus, the MeOH was removed after the extraction process by using a rotary evaporator. The enhancement effect was eliminated.

The chromatogram was shown in Figure 4. Thus, in this study, MeOH was used to extract the arsenic species from the seaweed, then the MeOH was removed to reduce the matrix interferences.

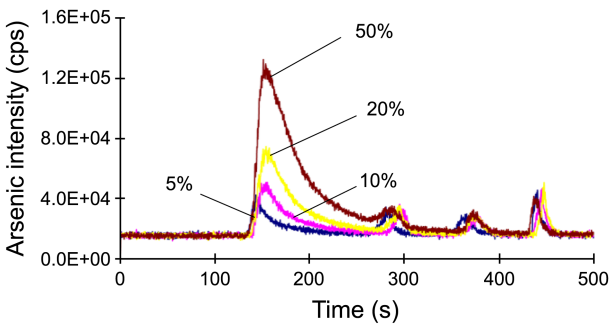

Figure 3. Chromatogram for arsenic species in MeOH solution.

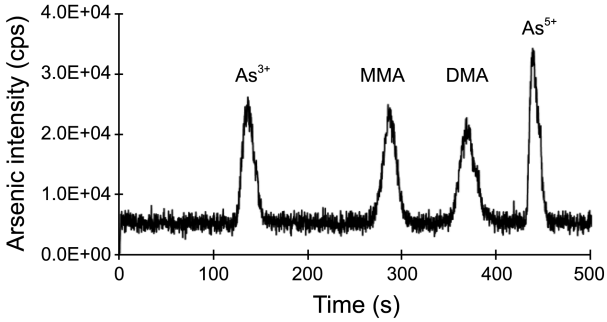

Figure 4. Chromatogram for arsenic species after removing MeOH.

The seaweed (sea mustard, laver, green laver and sea tangle) were investigated for the arsenic species. The chromatograms (Figure 5) were obtained from the seaweed. As the results

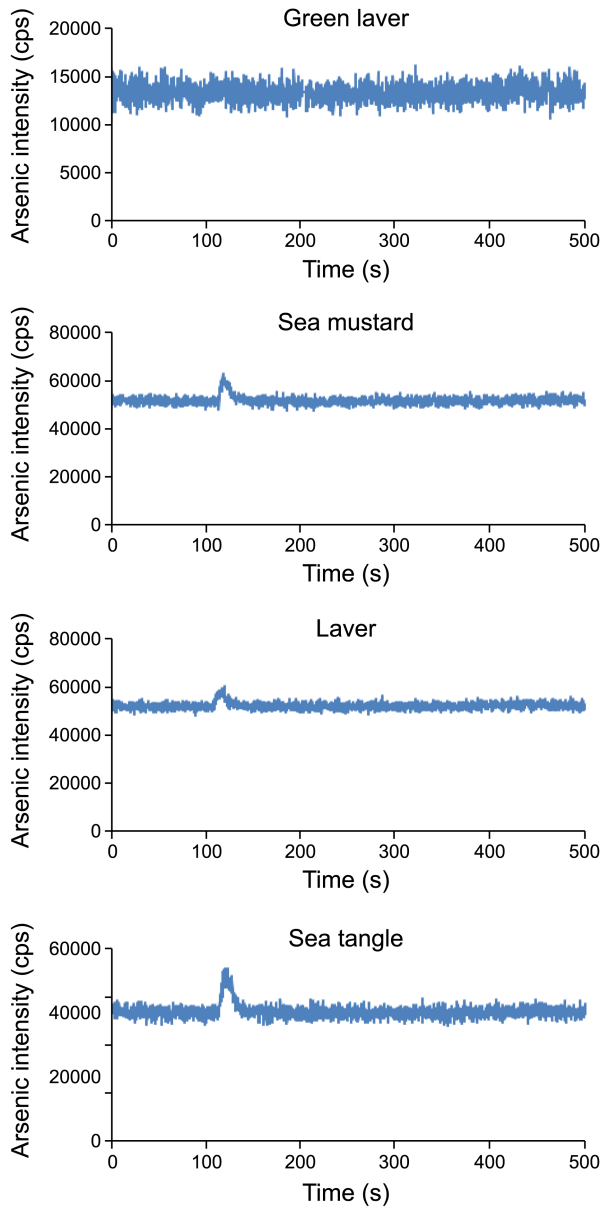

Figure 5. Chromatogram for arsenic species (As^{3+}, As^{5+}, MMA and DMA) in the seaweed.

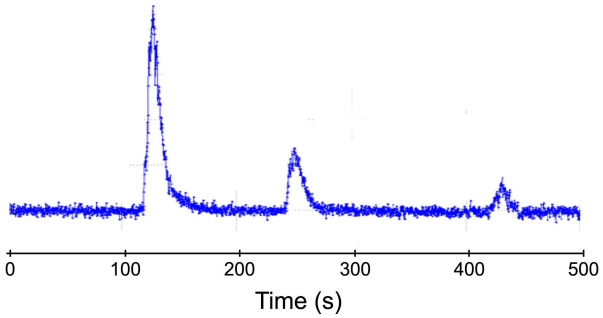

Figure 6. Chromatogram for As^{3+}, As^{5+}, MMA and DMA spiked in the green laver sample.

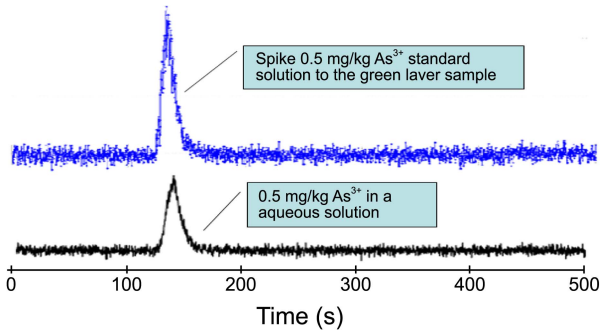

Figure 7. Chromatogram for As^{3+} spiked in the green laver sample.

are shown, the retention times for As^{3+}, As^{5+}, MMA and DMA were not clear. There are no peak in the chromatogram of Green Laver and only one peak in those obtained for the Sea Mustard, Laver and Sea Tangle.

To identify the arsenic species in seaweed, a standard solution of 0.5 mg/kg arsenic species (As^{3+}, As^{5+}, MMA and DMA) was spiked to the green laver, and the sample was pretreated using the same extraction method. The chromatogram was shown in Figure 6. It was still difficult to identify the arsenic species from the Figure 6. Each arsenic species was spiked to the green laver. First, As^{3+} was spiked, the chromatogram was shown in Figure 7. As the result was shown, the As^{3+} was not changed. Next, As^{5+} was spiked. As the chromatogram was shown in Figure 8, the As^{5+} was partially reduced to As^{3+}. Last, MMA and DMA were spiked to the green laver, separately. The chromatograms were shown in Figure 9 and Figure 10, the retention times of MMA and DMA were changed. The reasons might be due to the

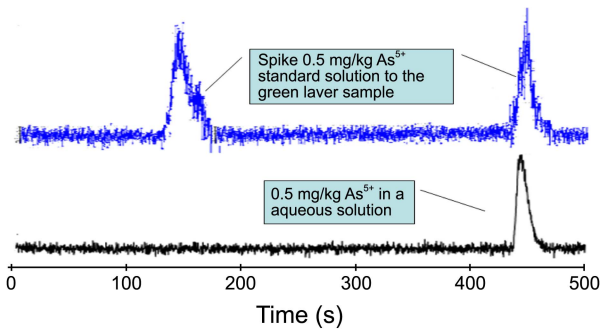

Figure 8. Chromatogram for As^{5+} spiked in the green laver sample.

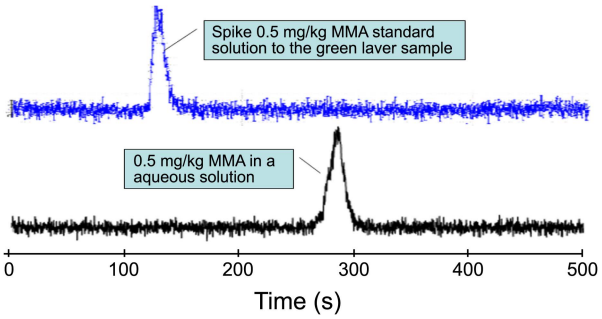

Figure 9. Chromatogram for MMA spiked in the green laver sample.

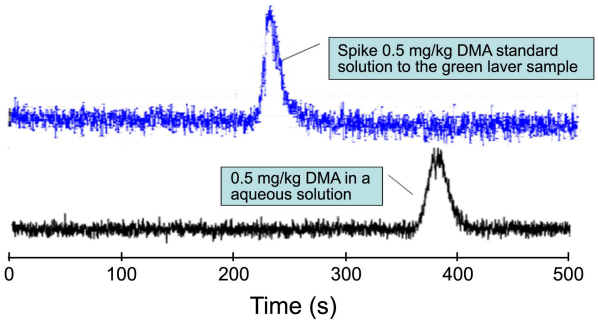

Figure 10. Chromatogram for DMA spiked in the green laver sample.

complex matrix in the seaweed.

Alginic acid was investigated for the matrix interference because the seaweed contains lots of alginic acid. A standard solution of 0.5 mg/kg arsenic species (As^{3+}, As^{5+}, MMA and DMA) was prepared using 1% alginic acid. The solution was pretreated using the extraction method for the arsenic species. The chromatogram was shown in Figure 11. As compare with Figure 6 and 11, the similar changes were found from the chromatograms. Thus, the alginic acid might be one of the matrix components in the seaweed that could affect the charge of the arsenic species. The results showed the MeOH extraction method and the chromatographic method was not applicable to seaweed samples for the speciation of arsenic.

Total Inorganic Arsenic. Another method was examined for the determination of inorganic species in the seaweed samples.[29] Ultrapure water (4.1 mL) and concentrated HCl

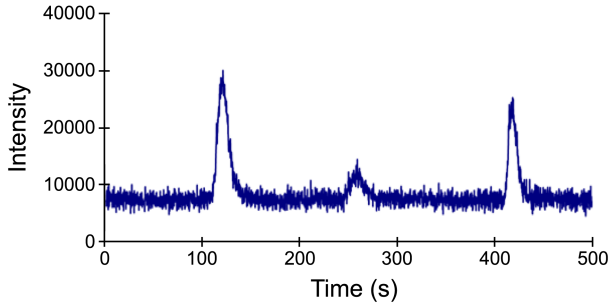

Figure 11. Chromatogram for arsenic species (As^{3+}, As^{5+}, MMA and DMA) in the 1% alginic acid solution.

Table 5. Results for the concentrations of inorganic arsenic in seaweed

	Concentration (mg/kg)
	Inorganic Arsenic (As^{3+} and As^{5+})
Sea Mustard	2.2 ± 0.1
Laver	0.6 ± 0.1
Green Laver	nd
Sea Tangle	2.9 ± 0.1

nd: not detected

(18.4 mL) were added to the 0.5000 g seaweed samples. The mixture was left overnight. The sample and extraction solvent were separated by using a centrifuge, and then the solution was filtered. HBr (2 mL) and 1.5% (w/v) hydrazine sulphate (1 mL) were added to reduce As^{5+} to As^{3+}. The inorganic arsenic was extracted into 30 mL of chloroform, then extracted into 20 mL of 1 M HCl.[30] The solvent was removed by a rotary evaporator to about 1 mL. The evaporation flask was washed with the ultrapure water. The solution was transferred to a 50 mL falcon tube, and the final volume was made to 20 mL.

We did recovery test for a standard solution of 0.5 mg/kg arsenic species (As^{3+}, As^{5+}, MMA and DMA). The sample solution was pretreated using the method described above. The recovery efficiency was shown as 113%. The seaweed samples were prepared by the pretreatment method. Then, the inorganic arsenic in the seaweed was determined by ICP-AES. The results were shown in Table 5. The concentrations of total inorganic arsenic in the sea mustard and sea tangle were higher than those of laver and green laver, and no inorganic arsenic was found in the green laver.

Conclusion

For the determination of total arsenic, the method (10% HNO_3 as extraction solvent + shake for 24 h) was used as a pretreatment method. The concentrations of total arsenic in sea mustard, laver, green laver and sea tangle were 52.1 ± 0.1, 15.6 ± 0.5, 3.62 ± 0.15 and 52.0 ± 0.1 mg/kg, respectively.

For the determination of inorganic arsenic, when HNO_3 was used for extraction solvent, the arsenic species could not be separated by the chromatographic method. Thus, HNO_3 was not suitable for the extraction solvent. When 50% methanol was used as extraction solvent, the retention times of MMA and DMA were changed, and As^{5+} was partially reduced to As^{3+}. Therefore, methanol could not be used as the extraction solvent for seaweed.

The quantitation of inorganic arsenic in seaweed was carried out by using chloroform and hydrochloric acid as extraction solvent. The concentrations of inorganic arsenic in sea mustard, laver and sea tangle were 2.2 ± 0.1, 0.6 ± 0.1 and 2.9 ± 0.1 mg/kg, respectively. The inorganic arsenic in green laver was not detected.

Acknowledgments. This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant number: 2011-0023778).

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