Promulgated, Jun 27, 2022
MOHWH0034.00

Method of Test for Inorganic Arsenic in Foods

1. Scope

This method is applicable for the determination of inorganic arsenic in seaweed, rice, aquatic animal, and fish oil.

2. Method

After extraction and oxidation, inorganic arsenic is determined by liquid chromatography/inductively coupled plasma mass spectrometry (LC/ICP-MS).

2.1. Equipment

2.1.1. Liquid chromatograph/inductively coupled plasma mass spectrometer.

2.1.1.1. Column: PRP-X 100, 5 μm, 4.6 mm i.d. × 15 cm, or an equivalent product.

2.1.2. Acid steam cleaning system.

2.1.3. Mill: Stainless steel, 1 mm mesh size.

2.1.4. Blender.

2.1.5. Vortex mixer.

2.1.6. Ultrasonicator: with a water bath and an automatic temperature controller, capable of controlling temperature at ± 4°C.

2.1.7. Centrifuge: centrifugal force > 3000 ×g.

2.1.8. pH meter.

2.2. Chemicals

Nitric acid, ultrapure grade (67-70%) and reagent grade;
Hydrogen peroxide (30%), ultrapure grade;
Glacial acetic acid, ultrapure grade;
Methanol, HPLC grade;
n-Hexane, HPLC grade;
Ammonium carbonate, reagent grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Arsenite and arsenate(Note), 1000 μg/mL, reference standards, ICP grade.

Note: The arsenite reference standard will be oxidized to arsenate and quantified as arsenate in this method. The arsenate reference standard is used for chromatographic reference control.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask(Note): 20 mL, 50 mL and 100 mL.

2.3.3. Membrane filter: 0.22 μm, PVDF.

Note: After cleaning, use an acid steam cleaning system to clean the apparatus with nitric acid (reagent grade) vapor for 2 hours, or soak the apparatus in nitric acid (reagent grade): water (1:1, v/v) overnight. Take the apparatus out, wash away the residual nitric acid with deionized water and dry.

2.4. Reagents

2.4.1. 50% acetic acid

Dilute 50 mL of glacial acetic acid with deionized water to 100 mL.

2.4.2. 1% (w/w) nitric acid containing 0.2 M hydrogen peroxide

Mix 20.4 mL of hydrogen peroxide(Note) and 500 mL of deionized water, add 10 mL of nitric acid (ultrapure reagent) slowly, and dilute with deionized water to 1000 mL. Prepare freshly before use.

Note: The hydrogen peroxide should be refrigerated immediately after use to prevent degradation.

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 19.2 g of ammonium carbonate with 800 mL of deionized water. Add 30 mL of methanol, adjustment to pH 8.5 with 50% acetic acid, and dilute with deionized water to 1000 mL. Filter with a membrane filter.

2.5.2. Solvent B

Transfer 2.5 mL of solvent A, add 30 mL of methanol, mix well, and dilute with deionized water to 1000 mL. Filter with a membrane filter.

2.6. Standard solution preparation

Accurately transfer 1 mL of arsenite reference standard, and dilute with 1% (w/w) nitric acid containing 0.2 M hydrogen peroxide to 100 mL as the standard stock solution. Store in the refrigerator. When to use, take appropriate volume of the standard stock solution, and dilute to 0-25 ng/mL with 1% (w/w) nitric acid containing 0.2 M hydrogen peroxide as the standard solutions.

2.7. Sample solution preparation

Transfer about 1 g of the homogenized sample accurately weighed into a centrifuge tube, add 10 mL of 1% (w/w) nitric acid containing 0.2 M hydrogen

peroxide, vortex-mix, and ultrasonicate for 30 min in a water bath at 80℃. After cooling, centrifuge at 3000 ×g for 10 min, and collect the supernatant. Add 5 mL of 1% (w/w) nitric acid containing 0.2 M hydrogen peroxide to the residue, and repeat the procedure described above. Combine the supernatants, and dilute with 1% (w/w) nitric acid containing 0.2 M hydrogen peroxide to 20 mL. Filter with a membrane filter, and take the filtrate as the sample solution(Note). Take an empty centrifuge tube, add 10 mL of 1% (w/w) nitric acid containing 0.2 M hydrogen peroxide, and perform the same procedure described above as the blank solution.

Note: If the sample solution of fish oil were found to contain residue oil, add 1 mL of *n*-hexane to the sample solution for liquid-liquid extraction. After separating the two phases, transfer the bottom layer for further analysis.

2.8. Identification and quantification

Accurately inject 20 μL of the sample solution, the blank solution and the standard solutions into the LC/ICP-MS separately, and operate according to the following conditions. Identify inorganic arsenic based on the retention time. Calculate the amount of inorganic arsenic in the sample by the following formula (mg/kg)(Note):

$$\text{The amount of inorganic arsenic in the sample (mg/kg)} = \frac{(C - C_0) \times V}{M \times 1000}$$

Where,

C：the concentration of the arsenate in the sample solution calculated by the standard curve (ng/mL)

C_0：the concentration of arsenate in the blank solution calculated by the standard curve (ng/mL)

V：the final make-up volume of the sample (mL)

M：the weight of the sample (g)

Note: Inorganic arsenic includes arsenite and arsenate. Arsenite in the sample is oxidized to arsenate by this method, so the amount of inorganic arsenic in the sample is expressed as arsenate.

LC/ICP-MS operating conditions(Note)

Column: PRP-X 100, 5 μm, 4.6 mm i.d. × 15 cm.

Column temperature: 25°C.

Flow rare: 1 mL/min.

Mobile phase: a gradient program of solvent A and B is as follows.

Time (min)	A (%)	B (%)
0 → 2	0 → 0	100 → 100
2 → 3	0 → 50	100 → 50
3 → 8	50 → 50	50 → 50
8 → 9	50 → 100	50 → 0
9 → 12	100 → 100	0 → 0
12 → 13	100 → 0	0 → 100
13 → 15	0 → 0	100 → 100

Radiofrequency power: 1550 W.

Plasma argon flow rate: 15 L/min.

Auxiliary argon flow rate: 0.9 L/min.

Detection mode: interference removal by gas.

Atomic mass (*m/z*): arsenic, 75.

Note: 1. The liquid chromatographic conditions used should effectively separate inorganic arsenic from organic arsenic.

2. All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. The limits of quantification for inorganic arsenic in seaweed, rice, aquatic animal and fish oil are all 0.02 mg/kg.
2. Further validation should be performed when interfering compounds appear in samples.

Reference

1. Chu, Y. L. and Jiang, S. J. 2011. Speciation analysis of arsenic compounds in edible oil by ion chromatography-inductively coupled plasma mass spectrometry. J. Chromatogr. A 1218: 5175-5179.
2. Narukawa, T., Chiba, K., Sinaviwat, S. and Feldmann, J. 2017. A rapid monitoring method for inorganic arsenic in rice flour using reversed phase-high performance liquid chromatography-inductively coupled plasma mass spectrometry. J. Chromatogr. A 1479: 129-136.

Reference chromatogram

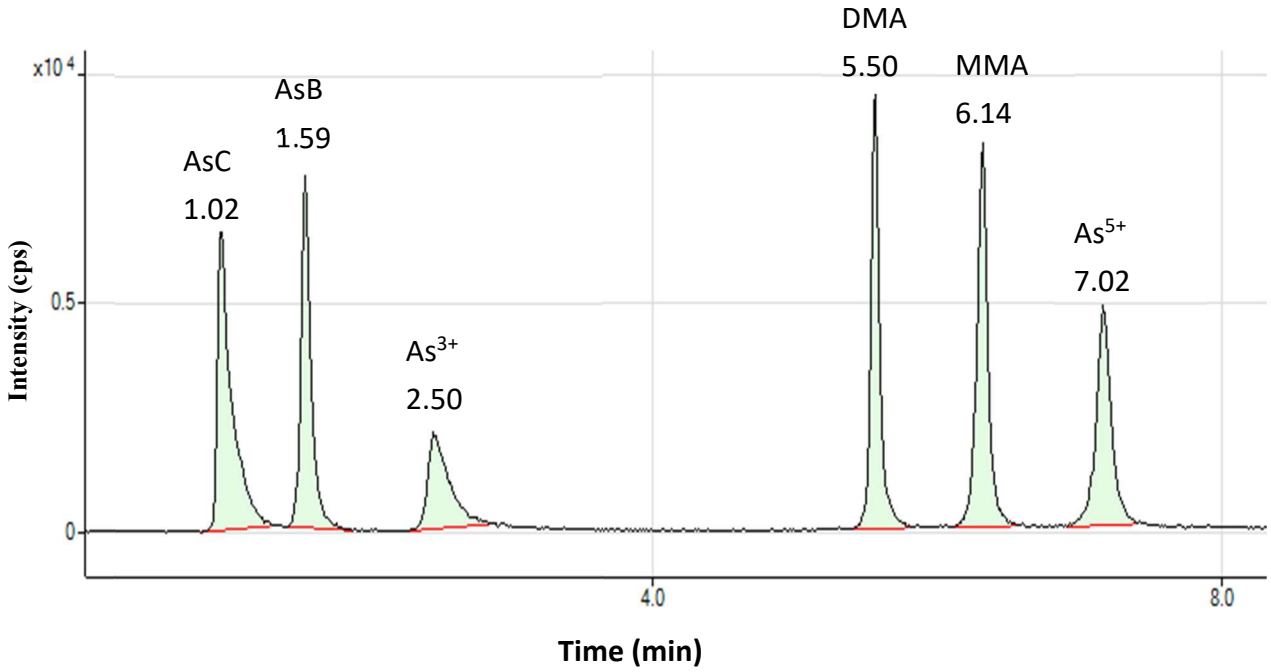

Figure. Chromatogram of arsenite, arsenate and organic arsenic standards analyzed by LC/ICP-MS.
AsC, arsenocholine; AsB, arsenobetaine; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid.