Method of Test for Methyl Mercury in Foods (3)

1. Scope

This method is applicable to the determination of methyl mercury in aquatic animals.

2. Method

After sample extraction, methyl mercury is determined by liquid chromatography/inductively coupled plasma mass spectrometry (LC/ICP-MS).

2.1. Equipments

2.1.1. Liquid chromatograph/inductively coupled plasma mass spectrometer.

2.1.1.1. Column: Synergi^{TM} Hydro-RP 80Å C18, 4 μm, 4.6 mm i.d x 15 cm, or an equivalent product.

2.1.2. Acid steam cleaning system.

2.1.3. Ultrasonicator: with water bath, capable of controlling temperature at ± 4°C.

2.2. Chemicals

Nitric acid, reagent grade;

L-Cysteine, reagent grade;

L-Cysteine hydrochloride monohydrate, reagent grade;

Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);

Chloromethyl mercury (1000 μg/mL), reference standard.

2.3. Apparatus

2.3.1. Extraction tube: 50 mL, PP.

2.3.2. Volumetric Flask$^{(note)}$: 50 mL, Borosilicate.

2.3.3. Vial: 2 mL, Brown, Borosilicate.

2.3.4. Membrane filter: 0.45 μm, PVDF.

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

2.4. Extraction solution preparation

Dissolve and dilute 10 g of L-cysteine hydrochloride with deionized water to 1000 mL(note).

Note: The extraction solution should be freshly prepared and used within 24 hours.

2.5. Mobile phase preparation

Dissolve and dilute 1 g of L-cysteine and 1 g of L-cysteine hydrochloride with deionized water to 1000 mL. Filter with a membrane filter.

2.6. Standard solution preparation

Accurately take appropriate amount of chloromethyl mercury reference standard, and dilute equivalent to 10 μg/mL methyl mercury with deionized water as the stock standard solution. When to use, dilute appropriate amount of the standard stock solution with the extraction solution to 0.4 ~ 20 ng/mL as the standard solutions.

2.7. Sample solution preparation

Transfer about 0.5 g of the homogenized sample accurately weighted into a extraction tube, add 45 mL of the extraction solution, shake vigorously for 10 sec by hands, and ultrasonicate for 40 min in a water bath at 60℃. After cooling to room temperature, dilute with the extraction solution to 50 mL, and filter with a membrane filter. Take the filtrate as the sample solution. Take a blank extraction tube, add 45 mL of the extraction solution, and perform the same procedure described above as the blank solution.

2.8. Identification and quantification

Accurately inject 50 μL of the sample solution and the standard solutions into the LC/ICP-MS separately, and operate according to the following conditions. Identify methyl mercury based on the retention time and the ratio of the peak area of ^{200}Hg to that of ^{202}Hg(note1). Calculate the amount of methyl mercury in the sample by the following formula:

$$\text{The amount of methyl mercury in the sample (ppm)} = \frac{(C - C_0) \times V}{W \times 1000}$$

Where,

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

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

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

W: the weight of sample (g)

LC/ICP-MS operating conditions(note 2)

LC conditions:

Column: Synergi™ Hydro-RP 80Å C18, 4 µm, 4.6 mm i.d x 15 cm.

Column temperature: 25°C.

Mobile phase: prepared according to section 2.5.

Flow rate: 1.0 mL/min.

ICP-MS conditions:

Radiofrequency power: 1550 W.

Plasma argon flow rate: 15 L/min.

Auxiliary argon flow rate: 0.9 L/min.

Nebulizer argon flow rate: 1.0 L/min.

Atomic mass: quantitation: 202 *m/z*; qualitation: 200 *m/z*.

Note: 1. The ratio of the peak area of ^{200}Hg to that of ^{202}Hg should be within 1.2 ~ 1.4.

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

Remark

1. The limit of quantitation for methyl mercury is 0.04 ppm.
2. Further validation shall be done when interference compounds appear in samples.

Reference

1. USFDA. 2008. Elemental analysis manual for food and related products.

4.8. High performance liquid chromatographic inductively coupled plasma mass spectrometric determination of methyl mercury and total mercury in seafood.

2. Hight, S. C. and Cheng, J. 2006. Determination of methyl mercury and estimation of total mercury in seafood using high performance liquid chromatography (HPLC) and inductively coupled plasma-mass spectrometry (ICP-MS): method development and validation. Anal. Chim. Acta 567: 160-172.