



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 5009.198-2016

**National food safety standard - Shellfishes - Test method of
domoic acid in amnesic shellfish poisoning**

食品安全国家标准 贝类中失忆性贝类毒素的测定

(English Translation)

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1 Scope

This Standard specifies enzyme-linked immunosorbent assay, liquid chromatography, and liquid chromatography-tandem mass spectrometry for the determination of amnesic shellfish poisoning in shellfish.

The enzyme-linked immunosorbent assay in this Standard applies to the determination of amnesic shellfish poisoning in shellfish and its products. Liquid chromatography and liquid

chromatography-tandem mass spectrometry apply to the determination of domoic acid (DA) in amnesic shellfish poisoning in shellfish and its products (excluding salted products).

Enzyme-linked immunosorbent assay

2 Principle

The basis of this method is a competitive enzyme-linked immunoreaction. The

ELISA plate is coated with a capture antibody against the domoic acid antibody.

ADD anti-domoic acid antibody, standard solution or sample solution, and domoic acid enzyme marker. The free amnesic shellfish poisoning competes with the domoic acid enzyme marker for the domoic acid antibody, while the domoic acid antibody is linked to the capture antibody. The unbound enzyme marker is removed in the washing step. The enzyme matrix and developer are added to the micropores and incubated. The bound enzyme marker converts the colorless color former into a blue product. After the reaction stop solution is added, the color is changed from blue to yellow. At 450 nm, MEASURE the absorbance value of the microporous solution. The content of amnesic shellfish poisoning in the sample is inversely proportional to the absorbance value and quantitatively calculated according to the drawn standard curve.

3.2.5 Eluent: PIPETTE 0.5 mL of tween-20; USE PBS solution (pH7.4) to dilute
to 1000 mL.

3.2.6 Sulfuric acid solution (6 mol/L): PIPETTE 319.2 mL of sulfuric acid; ADD
slowly it to 600 mL of water; USE water to dilute to 1000 mL.

3.3 Standard

Domoic acid (DA, C₁₅H₂₁NO₆, CAS No. 14277-97-5) standard solution.

3.4 Preparation of standard solutions

DA standard series working solutions: PIPETTE accurately appropriate amount of DA standard solution; USE PBS solution to dissolve and dilute, to prepare the DA standard series working solutions with mass concentrations of 0 ng/mL,

0.5 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 5.0 ng/mL, and 10.0 ng/mL, respectively.

They are used right after they are prepared.

3.5 Materials

3.5.1

Microporous plate coated with DA capture antibody.

Note: Commercialized kits, if the technical parameters for evaluation meet the requirements of this Standard, are also suitable for this Standard. See Appendix A.

3.5.2

Aqueous-phase microporous filter membrane

0.45 μm .

4 Instruments and equipment

4.1

Microplate reader.

4.2

Balance

The sensitivity is 0.01 g.

4.3

Homogenizer.

4.4

Centrifuge

Speed ≥ 6000 r/min.

5.1 Sample collection

COLLECT at least 10 shellfish samples and make the shellfish meat more than

200 g. The frozen sample is placed in a heat preservation box for freezing inspection; or it is guaranteed to be in a low temperature state (0 °C~10 °C) for inspection. For a shelled sample, it shall be opened, and after removing the water, frozen for inspection.

microporous rack and labeled, including blank control pore, standard solution pore, and sample solution pore. MAKE parallel pores respectively. ADD 150 μL of PBS solution (pH7.4) to the blank control pore. ADD 50 μL of amnesic shellfish poisoning standard series working solution to the standard solution pore. ADD 50 μL of the sample solution to the sample solution pore. ADD 100

μL of DA enzyme marker working solution to all the above micropores and mix gently; ADD 100 μL of DA antibody working solution and rapidly mix well for 1 min. USE viscose paper to seal the micropores to prevent evaporation of the solutions; incubate at 4 °C for 2 h. After

the incubation, pour out the liquid from the pores; inject 300 μL of eluent into each micropore to rinse; overturn the microporous plate; pour out the liquid from the pores; repeat the above plate- washing operation twice and pat dry on the absorbent paper. ADD 150 μL of hydrogen peroxide and TMB to each pore and mix well; incubate at room temperature for 30 min in the dark. ADD 50 μL of sulfuric acid solution (6 mol/L) to each pore and mix rapidly; terminate the reaction; measure in 30 min and record the absorbance value at a wavelength of 450 nm. If the sample diluent is measured beyond the linear range of the standard curve, the dilution factor may be expanded and the determination repeated.

5.5 Making of standard curve

USE the base 10 logarithm of the mass concentration of amnesic shellfish poisoning standard series working solution as the abscissa. USE the percentage absorbance value of standard solution calculated according to formula (1) as the ordinate. DRAW the standard curve.

The percentage absorbance value of amnesic shellfish poisoning standard solution (or sample solution) is calculated according to formula (1):

Where:

A - Percent absorbance value;

S - Average absorbance value of amnesic shellfish poisoning standard working solution or sample solution;

S1 - Average absorbance value of blank control pores;

S0 - Average absorbance value of 0 $\mu\text{g/L}$ amnesic shellfish poisoning standard working solution.

9 Reagents and materials

Unless otherwise specified, the reagents used in this method are chromatographically pure; the water is Grade 1 water specified in GB/T 6682.

9.1 Reagents

9.1.1

Acetonitrile (CH_3CN).

9.1.2

Methanol (CH_3OH).