



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

GB 5009.212-2016

**National food safety standard - Determination of Diarrheic
Shellfish Poison in Shellfish**

食品安全国家标准 贝类中腹泻性贝类毒素的测定

(English Translation)

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1 Scope

This Standard specifies mouse biology method, enzyme linked immunosorbent assay (ELISA method) and liquid chromatography - tandem mass spectrometry for the determination of diarrheic shellfish poison in shellfish.

In this Standard, mouse biology method and enzyme linked immunosorbent assay (ELISA

method) are applicable to the determination of diarrheic shellfish poison in shellfish and shellfish products; liquid chromatography - tandem mass spectrometry is applicable to the determination of diarrheic shellfish poison okadaic acid (OA), dinophysistoxins-1 (DTX-1) and dinophysistoxins-2 (DTX-2) in edible part of shellfish and shellfish products (excluding salted products).

Mouse Biology Method

2 Principle

Use acetone to extract diarrheic shellfish poison (DSP) in shellfish. Through the distribution with anhydrous ether, decompression and evaporation, take saline solution, which contains 1% Tween-60, as the dispersing medium, prepare DSP injectable suspension. Inject the injectable suspension into mouse's peritoneal cavity. Observe the survival of the mouse; calculate its virulence.

3 Reagents and Materials

Unless it is otherwise stipulated, all reagents used in this Method shall be analytically pure. Water shall be Grade-1 water stipulated in GB/T 6682.

3.1 Reagents

3.1.1

Acetone (C₃H₆O).

3.1.2

Anhydrous ether (C₄H₁₀O).

3.1.3

Tween-60 (C₆₄H₁₂₆O₂₆).

3.1.4

Sodium chloride (NaCl).

5.2.2 Frozen sample

At room temperature, thaw frozen sample, till it displays semi-frozen state. In terms of shelled frozen sample, in accordance with the methods in 5.2.1, wash, open the shell, rinse and take out the shell meat; remove frozen pieces attached to the external part of the shell meat; wipe off the moisture. Cut the shell meat into pieces.

5.3 Sample Extraction

Weigh-take 200 g of shell meat sample, which is already cut into pieces; place it in a homogeneous cup. In accordance with the volume ratio, add a triple amount of acetone, then, homogenize it for over 2 min. Pour the well-homogenized substance into a Brinell funnel, then, extract and filter it; gather the filtrate. Respectively use acetone, which is 2 times the amount of the residue, to rinse the residue twice. Combine the filtrate and the above-mentioned filtrate. Transfer the filtrate into a 500 mL of round-bottomed flask.

At $56\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, decompress and concentrate it to remove acetone, till greasy substance is separated from the liquid surface.

Use 100 mL ~ 200 mL of anhydrous ether to dissolve the greasy substance; pour it into a separating funnel. Use a small amount of anhydrous ether to rinse the round-bottomed flask; combine them and pour them into the separating funnel. Use a small amount of water to wash the lower sticky wall part. Slightly oscillate (avoid generating emulsion); place it still for stratification. Then, remove the aqueous layer (the lower layer).

Use water, which is equivalent to half the amount of ether, to wash the ether layer twice.

Remove the aqueous layer, then, transfer the ether layer into a 250 mL or 500 mL round-bottomed flask. At $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, decompress and concentrate it to remove ether.

Use a small amount of anhydrous ether to transfer the concentrated substance into a 50 mL or 100 mL round-bottomed flask. Again, decompress and concentrate it to remove ether.

Use 1% Tween-60 saline solution to transfer all the concentrated substance into a scale test tube, then, dilute it into 10 mL. Thoroughly shake it to prepare a homogenous suspension. 1 mL of the suspension is equivalent to 20 g of sample. Take this suspension as test stock solution.

Use the test stock solution to inject mice. When within 24 h, 2 or 3 mice died, oscillate the test stock solution, so that it could become homogenous suspension, then, use 1%

Tween-60 saline solution to step-by-step dilute it, in accordance with Table 1, into 4 times or 16 times of diluent. Thoroughly mix it up. Then, in accordance with Table 1, inject mice.

5.4 Mouse Test

Select 6 healthy ICR strain of male mice, weight: 16 g ~ 20 g. Randomly divide them into two groups, namely, test group and solvent control group; 3 mice in each group.

Enzyme Linked Immunosorbent Assay (ELISA method)

7 Principle

In accordance with competitive enzyme-linked immunoreactivity, dissociative diarrheic shellfish poison compete with its enzyme marker for diarrheic shellfish poison antibody.

Unbonded enzyme marker is eliminated in the step of rinsing. Add enzyme substrate and color developing reagent to the hole and incubate it. The bonded enzyme marker converts colorless color former into blue product. After adding reaction termination fluid, the color turns from blue to yellow. Under the wavelength of 450 nm, use ELIASA to measure the absorbance value of microporous solution. The content of diarrheic shellfish poison in the sample is inversely proportional to the absorbance value. In accordance with the standard curve being drawn, conduct quantitative calculation.

8 Reagents and Materials

Unless it is otherwise stipulated, all reagents used in this Method shall be analytically pure. Water shall be Grade-1 water stipulated in GB/T 6682.

8.1 Reagents

8.1.1

Methanol (CH_3OH).

8.1.2

Disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$).

8.1.3

Sodium chloride (NaCl).

8.1.4

Potassium chloride (KCl).

8.1.5

Potassium dihydrogen phosphate (KH_2PO_4).

8.1.6

Tween-20 ($\text{C}_{58}\text{H}_{114}\text{O}_{26}$).

8.1.7

Bovine serum albumin (BSA).

8.1.8

Enzyme marker.