



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

GB/T 5009.206-2007

Replacing GB/T 23217-2008; GB/T 5009.206-2007; SN/T 1569.2-2013

**Determination of tetrodotoxin in aquatic products --
HPLC-fluorescence detection method**

鲜河豚鱼中河豚毒素的测定

(English Translation)

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Foreword

Instead of the standard GB/T 23217-2008 "aquatic tetrodotoxin liquid chromatography - fluorescence detection method", GB/T 5009.206-2007 "Determination of tetrodotoxin in puffer fish fresh" and SN/T 1569.2-2013 "puffer fish tetrodotoxin outlet Detection Method 2 Point. mouse bioassay. " as compared with the present standard GB/T 5009.206-2007, major changes are as follows.

--- standard name was changed to "National Food Safety Standard Determination of tetrodotoxin in aquatic products";

--- an increase of purification steps;

--- modified enzyme-linked immunosorbent assay.

National Food Safety Standard Determination of aquatic tetrodotoxin

1 Scope

This standard specifies the method for the determination of tetrodotoxin in aquatic products.

The standard method for the determination of a first puffer fish muscle, liver, skin and gonad tissues tetrodotoxin. The second method is suitable for puffer fish Muscle, liver measured, tetrodotoxin skin and gonads. The third method is suitable for puffer fish, snails, shrimp, oysters, squid, and Clam Determination of tetrodotoxin. The fourth method is suitable for determination of puffer fish muscle, liver, skin and gonad tissues tetrodotoxin.

The first mouse bioassay method Principle 2 the principle of tetrodotoxin soluble in an acidic solution, the sample was prepared acetic acid solution twice (0.5%) boiling extraction, centrifugation 20000g the supernatant was collected and the two supernatants were combined volume to 25mL, a mouse bioassay. The mice died after injection of the sample extract Time, isolated rat Units and weight correction unit mice rats, calculated to determine the content of tetrodotoxin.

3 Reagents and materials

Unless otherwise indicated, the methods used were of analytical grade reagents, water was used three predetermined water GB 6682.

3.1 Reagent

3.1.1

glacial acetic acid (CH_3COOH).

3.1.2

sodium hydroxide (NaOH).

3.2 Reagent preparation

3.2.1

acetic acid (0.5%). 5mL of glacial acetic acid diluted with water to 1L.

3.2.2

sodium hydroxide solution (1mol/L). 40g of sodium hydroxide dissolved in water and dilute to 1000mL.

3.3 Standard

Tetrodotoxin ($\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_8$, CAS number. 4368-28-9), purity $\geq 98\%$, or certified by the state and granted a certificate of standard reference materials Reference material.

Boiling water bath for 10min, with constant stirring to prevent clumping tissue. Cooled to room temperature, 20000g high-speed centrifugal 20min, supernatant was pipetted 25mL volumetric flask. Acetic acid solution was added to the precipitate (0.5%) 11mL, mix thoroughly, boiled in a boiling water bath for 5min, stirring constantly to prevent Only organization agglomeration. Cooled to room temperature, 20000g high-speed centrifugal 20min, the supernatant was pipetted. The combined supernatant was mixed with a solution of acetic acid (0.5%) volume to 25mL. This extract is equivalent to 0.4g 1mL sample.

5.2.2 Liver tissue samples

Accurately weighed 10g (to the nearest 0.1g) in 50mL centrifuge sample tube, was added acetic acid (0.5%) 8mL, mix well, at room temperature High speed centrifugation at 20000g 20min, supernatant was pipetted 25mL volumetric flask. 8mL extracted repeatedly with acetic acid (0.5%) 1 time. The combined supernatant was mixed with a solution of acetic acid (0.5%) volume to 25mL. This extract is equivalent to 0.4g 1mL sample.

5.2.3 Skin tissue sample

Accurately weighed 10g (to the nearest 0.1g) in 50mL centrifuge sample tube, was added acetic acid (0.5%) 11mL, mix well, chamber High speed centrifugation at 20000g temperature 20min, supernatant was pipetted 25mL volumetric flask. The extraction was repeated with 11mL acetic acid (0.5%) 1 time. The supernatant was combined with a

solution of acetic acid (0.5%) volume to 25mL. This extract is equivalent to 0.4g 1mL sample.

Note 1: The samples were prepared, frozen samples need to be operated in a semi-thawed state. If not timely test sample, should be stored at 4 °C, tested within 24h.

Note 2: If the case where the puffer fish organs or tissue sample of less than 10g, is extracted according to the above method of operation, need to reduce the amount of acetic acid (0.5%) added.

Note 3: extract should contain not be separated from the liquid surface of the oil was centrifuged, gum or lipids.

5.3 Mouse assay

Select 19.0g ~ 21.0g of specific pathogen free (SPF) male Kunming 6 healthy mice were weighed and body weight was recorded. Randomly divided of the experimental group and the control group two, three per group. Extract or acetic acid solution to each test mouse by intraperitoneal injection of 1mL sample (0.5%) (control solution). If overflow injection during injection, the mice should be discarded and a re-injected mice. Record Note Shooting start and finish time, carefully observe and record the time of death when the mice stopped breathing (to the last breath is only a mouse).

If the sample extract after injection, mice were less than the time of death 7min, press Appendix A Table A.1 calculated 1mL sample extract Virulence, as a reference, formulated ambassador mice died 7min ~ 13min required dilution, use solution of acetic acid (0.5%) in dilute Release, the diluent re-injected mice at least 3 to determine the virulence of the sample. Specific examples in Appendix B. If the sample solution injection, mice Time of death is greater than or equal to 7min, virulence is determined directly from the sample median lethal time.

Symptoms of poisoning death in mice. Mice were injected with tetrodotoxin quiet early, followed by difficulty in breathing, shortness of breath, abdominal contraction accelerated, Unresponsive, then sudden Rush, sprint jerks, flee, moved, flipped jumping operation like struggling, after several tens of seconds, mice Leg twitch violently, after 2 or 3 times to climb lying immobile, weak abdominal breathing gradually slowed down, and finally death. To stop breathing as a judgment of death standard.

6.1 Calculate virulence

The time to death of mice is obtained in the test, the median lethal time is calculated, and the calculated Table A.1 virulence (if the bit when the time is right in the middle of the number of lethal two times given in Table A.1, whichever is greater of the two values of the time period; if given in Table A.1 of between when the time out of the two, but not exactly in the middle, then take a time value closer to the median time to death). If the mouse body