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

GB 29682-2013

**Determination of Penicillin residues in aquatic products by
High Performance Liquid Chromatographic method**

食品安全国家标准 水产品中青霉素类药物多残留的测定 高效液相色谱法

(English Translation)

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1 Scope

This standard specifies the sample preparation and high performance liquid chromatographic method Seafood by penicillins residues detected.

This standard applies to edible fish tissues penicillin G, oxacillin, dicloxacillin, and nafcillin single or multiple drug residue Allowance of detection.

2 Normative references

The following documents for the application of this document is essential. For dated references, only applies to the version dated paper Pieces. For undated references, the latest edition (including any amendments) applies to this document.

GB/T 6682 Water for analytical laboratory specifications and test methods Principle 3 the sample of residual penicillin drugs, extracted with acetonitrile, an HLB column purified, 1,2,4-triazole derivatives and mercuric chloride solution, high performance liquid Chromatography - UV determination, external standard.

4 Reagents and Materials

The following reagents used, unless otherwise stated are analytical reagents, water should be consistent with a water GB/T 6682 provisions.

4.1

penicillin G, oxacillin, dicloxacillin, and nafcillin Standard. content $\geq 98\%$.

4.2

methanol.

4.3

acetonitrile.

Sodium thiosulfate pentahydrate 4.4.

Sodium dihydrogen phosphate dihydrate 4.5.

Disodium hydrogen phosphate anhydrous 4.6.

4.7

ammonium sulfate.

4.8

sodium hydroxide.

4.9

n-hexane.

4.10 1,2,4-triazole.

4.11

mercuric chloride (II).

4.12

HLB solid phase extraction column. 60mg/3mL, or equivalent person.

4.13

mobile phase A. sodium thiosulfate pentahydrate 3.9g, dissolved in water, adding sodium dihydrogen phosphate dihydrate 10.2g, disodium hydrogen phosphate anhydrous 4.9g, ammonium sulfate 6.8g, dissolved in water and diluted to 1000mL, membrane filtration.

4.14

mercuric chloride (II) solution. Take mercuric chloride (II) 0.27 g of, dissolved and diluted to 10mL with water, now with the current.

4.15 5mol/L sodium hydroxide solution. take sodium hydroxide 20g, dissolve and dilute to

100mL with water.

4.16

derivatizing reagents. 13.78 g 1,2,4-triazole-taking, 60mL water was dissolved, the solution was added mercuric chloride 10mL, with 5mol/L hydroxide Sodium solution was adjusted to pH 9.0, diluted with water to 100mL. 2 °C ~ 8 °C dark storage period of three months.

4.17 1mg/mL Penicillin standard stock solution. Weigh accurately penicillin G, oxacillin, dicloxacillin, and nafcillin the standards for each 10mg, respectively 10mL volumetric flask, dissolve and dilute to volume with water, is formulated at a concentration of 1mg/mL penicillin G, oxacillin, dicloxacillin, and nafcillin standard stock solution, aliquoted, immediately stored in the dark at -20 deg.] C or less, a period month. Repeated freezing and thawing should not use.

4.18 10µg/mL penicillin G standard working solution. precise amount of 1mg/mL penicillin G standard stock solution 1.0mL, in 100mL Volumetric flask, dissolve and dilute to volume with water, is formulated at a concentration of 10µg/mL penicillin G standard working solution and packaging, to immediately at -20 °C Stored in the dark, valid for one month. Repeated freezing and thawing should not use.

4.19 10µg/mL oxacillin, dicloxacillin and nafcillin working standard solutions. precise amount of 1mg/mL West oxacillin Lin, dicloxacillin and nafcillin 1.0 mL of each standard stock solution, to a 100mL volumetric flask, dissolve and dilute to volume with water, formulated at a concentration of 10µg/mL oxacillin, dicloxacillin and nafcillin working standard solutions, dispensing, to immediately at -20 °C Stored in the dark, valid for one month. Repeated freezing and thawing should not use.

5 Apparatus

5.1

HPLC. with UV detector.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

freezing high-speed centrifuge.

5.5

temperature water bath.

5.6

vortex mixer.

5.7

SPE.

5.8

tissue homogenizer.

5.9

nitrogen blow device.

5.10

centrifuge tube. 50mL.

5.11

filter. 0.45 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

To the scales of fish, peeled, taken along the back muscle tissue, muscle or skin take fish tissue, minced, and homogenized.

--- the test sample taken after homogenization, as the feed try.

--- blank sample taken after homogenization, as a blank sample.

--- blank sample taken after homogenization, adding a suitable concentration of the standard working solution, is added as a blank sample.

Save 6.2 sample

Or less at -20 °C.

Determination Step 7

7.1 Preparation of standard curve

They were the precise amount of 10 μ g/mL penicillin G standard working solution and 10 μ g/mL oxacillin, dicloxacillin and nafcillin mixed Standard working solution combined amount, diluted with mobile phase to prepare a penicillin G concentration of 20,50,100,200,400,800 and 1600 μ g/L, oxacillin West Lin, dicloxacillin and nafcillin concentrations 100,200,400,800,1600,3200 and 6400 μ g/L series penicillins Mixed standard solution, 500 L depicting, by derivatization steps for HPLC assay. Peak area was measured to the vertical axis, corresponding to the standard Standard solution concentration as the