

ICS 67.020
CCS C 53



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

GB 29702-2013

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

食品安全国家标准 水产品中甲氧苄啶残留量的测定 高效液相色谱法

(English Translation)

Issued on: September 16, 2013

Implemented on: January 1, 2014

**Issued by: Ministry of Agriculture of the People's Republic of China,
National Health and Family Planning Commission of the People's
Republic of China**

Table of Contents

1	Scope
2	Normative references
4	Reagents and materials
5	Apparatus
6.1	Preparation of the sample
7.1	Extract
7.2	Purification
7.3	Preparation of standard curve
7.4	Determination
7.5	Blank test
8	Results calculated
9.1	Sensitivity
9.2	Accuracy
9.3	Precision of the method

1 Scope

This standard specifies the sample preparation and high performance liquid chromatographic method trimethoprim residues in aquatic products.

This standard applies to fish (including eel), shrimp, crab and turtles Residues in edible tissues of trimethoprim.

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 SC/T 3016 aquatic sampling Principle 3 the specimens remaining trimethoprim, extracted with chloroform and an acidic methanol solution, back extracted with methylene chloride, the net MCX SPE of HPLC - UV determination, external standard.

4 Reagents and materials

The following reagents used, unless otherwise stated were of analytical reagent; water as a water line with GB/T 6682 provisions.

Trimethoprim 4.1 Standard. content $\geq 98\%$.

4.2

chloroform. HPLC grade.

4.3

Methanol. HPLC grade.

4.4

perchloric acid. pure class distinctions.

4.5

Sulfuric acid. excellent pure.

4.6

dichloromethane. chromatography.

4.7

Sodium hydroxide. pure class.

4.8

acetic acid. excellent pure.

4.9

ammonia.

4.10

MCX cation SPE. 60mg/3mL, or equivalent person.

4.11 0.5% perchloric acid solution. Take perchlorate 5mL, dissolved and diluted with water to 1000mL.

4.12 0.1mol/L sulfuric acid solution. Take 5.4mL of sulfuric acid, dissolved and diluted to 1000mL with water.

4.13 2mol/L Potassium hydroxide. potassium hydroxide to take 112.2g, dissolved and diluted with water to 1000mL.

4.14 5% acetic acid solution. Take 50mL acetic acid, dissolved and diluted with water to 1000mL.

4.15 5% ammonia in methanol. aqueous ammonia to take 5mL, dissolved in methanol and

diluted with 100mL.

4.16 100µg/mL trimethoprim standard stock solution. Weigh accurately trimethoprim standard 10mg, in 100mL flask with methanol Dissolved and diluted to the mark to prepare a stock standard solution trimethoprim concentration of 100µg/mL of. -4 °C or less from light, valid for 3 Months.

4.17 10µg/mL trimethoprim standard working solution. precise amount of trimethoprim 1.0 mL standard stock solution, to a 10mL volumetric flask, with methyl Alcohol diluted to the mark, formulated at a concentration of trimethoprim working standard solution 10µg/mL of. -4 °C or less from light, valid for 3 Months.

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

homogenizer.

5.5

vortex mixer.

5.6

centrifuge.

5.7

rotary evaporator.

5.8

SPE.

5.9

eggplant-shaped bottle.

5.10

stoppered centrifuge tube. 50mL.

5.11

separatory funnel. 150mL.

5.12

filter. 0.45 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

Fresh or thawed take appropriate blank or test 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

-18 °C below saved.

Determination Step 7

7.1 Extract

Sample Weigh $5g \pm 0.05g$, in 50mL stoppered centrifuge tube, add chloroform 15mL, methanol 14mL, 0.1mol/L sulfuric acid solution Solution 6mL, vortexed 2min, 4000r/min centrifugal 3min, 150mL supernatant to a separatory funnel. Methanol was added to the residue and 14mL 0.1mol/L sulfuric acid solution, 6 mL, extraction was repeated once, the two supernatants were combined in a separatory funnel, was added 2mol/L hydroxide Solution of potassium 2mL, dichloromethane 30mL, shake for 2min, standing layer, the lower layer was taken to the eggplant-shaped flask, added to a separatory funnel with dichloromethane 30mL extraction was repeated once, the lower two combined solution at 40 °C rotary evaporated to near dryness, washed with 5% acetic acid solution to dissolve the residue 6mL, spare.

7.2 Purification

MCX cation exchange column washed with methanol and 5% acetic acid solution 6mL 6mL activated, taking stock solution passed through the column, washed with 5% acetic acid solution and 6mL 6mL methanol, and eluted with ammonia in methanol 15mL, at 40 °C rotary evaporated to near dryness and dissolved with mobile phase 1.0mL the residue was filtered through a filter for the Determination.